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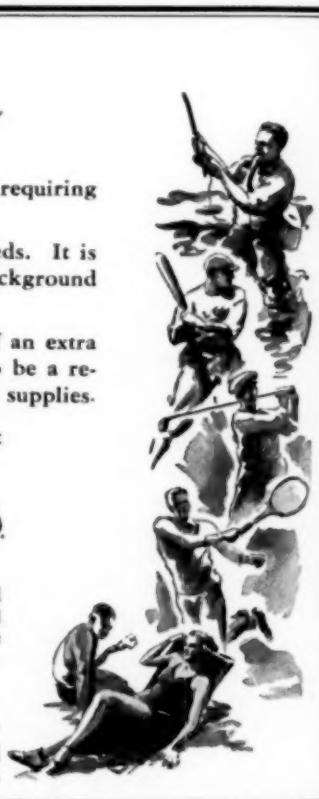

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The Assay of Ergot*

By Ralph G. Smith†

The purpose of this paper is to call attention to recent work, to point out certain general problems and to make certain suggestions in connection with the assay of galenical preparations of ergot.

Since the discovery in 1935 of ergonovine it has been advocated that the official U. S. P. method for ergot assay is inadequate. This method measures the total active alkaloids of ergot by the Cock's Comb method. The uterine-stimulating action of ergonovine is definitely stronger than that of the ergotoxine group, but the available evidence indicates that a difference of comparable degree is not reflected by the Cock's Comb reaction. The same objection holds for other methods such as the paradimethylaminobenzaldehyde colorimetric method and its modifications which depend on the lysergic acid radical common to both groups of alkaloids. Furthermore, the epinephrine-inhibiting action of ergonovine is so weak that the Broom-Clark method fails entirely to measure the content of this substance in ergot. If it could be assumed that the proportion of ergonovine to the other active alkaloids of ergot is constant in various samples, such criticism would not be valid. However, the evidence based on the isolation of pure alkaloids and on separate assays of water-soluble and water-insoluble fractions does not justify such an assumption. There are statements in the literature to the effect that some ergots contain no ergonovine (1, 2), while the results of certain workers (3, 4) indicate that as much as one-third of the total alkaloids of some ergots are water-soluble. Consequently, in order to assay the therapeutic activity of galenical preparations, definite modifications of the standard methods must be made, or new ones devised.

A logical procedure would seem to be the separation of ergonovine from the ergotoxine group prior to assay. Encouraging efforts in this regard have already been made by certain investigators, although further experience in various laboratories is necessary before the reliability of the suggested methods can be evaluated. Hampshire and Page (3), after removal of water-soluble alkaloids from the ether extract of ergot by dilute ammonia water, assayed the remaining insoluble alkaloids and also the original ex-

* Presented at the thirteenth meeting of the United States Pharmacopœial Convention, Washington, D. C., May 13–15, 1940.

† Laboratory of Pharmacology, University of Michigan Medical School, Ann Arbor.

tract containing all alkaloids, the ergonovine or soluble fraction being determined by difference. This method has also been used by others (5). Schou and Tønnesen (6) have effected separation of ergotoxine and ergonovine by ether extraction from aqueous or alcoholic aqueous solutions, the former alkaloid being extracted at a p_H of 4 and the latter at definitely alkaline reactions, the two groups of alkaloids then being assayed separately. Allport and Porter (4) also assayed the water-soluble fraction directly after its precipitation under definitely prescribed conditions by antimony trichloride. Trabucci (7) has accomplished separation by precipitation of ergotoxine with picric acid, leaving the ergonovine in solution, a procedure which has also been applied by Christensen and Reese (8). Two of the above groups of workers (3*b*, 4) have reported little success with the picric acid method, apparently due to incomplete precipitation of ergotoxine, although the actual results recorded by Hampshire and Page (3*b*) appear promising. The assay of the fractions has been carried out in all of this work by the *p*-dimethylaminobenzaldehyde colorimetric method. This method, which is official for the British Pharmacopœia, of course measures total rather than only biologically active alkaloids. It would be theoretically valid if inactive isomers were not present at all, or were present in always the same proportion with the active group, a premise for which there is no evidence. However, it was apparently adopted on the assumption that its superiority to biological methods with regard to experimental error and possibly simpler procedure outweighed its theoretical deficiencies.

It is possible that a biological method assaying only active alkaloids might be similarly applied after separation of water-soluble and water-insoluble principles, and in fact Oettel and Bachmann (9) have reported a procedure for the assay of ergonovine in ergot preparations after precipitation of ergotamine. It concerns the uterine response to intramuscular injections of the drug as recorded from a balloon inserted into the puerperal uterus of a cat or rabbit. Such a preparation, in their hands, has proved extremely sensitive to ergonovine and relatively insensitive to ergotamine so that traces of the latter still present have a negligible effect.

Undoubtedly certain difficulties will be encountered in attempting to apply biological methods to fractions of ergot. It is advisable to keep the following points in mind if possibilities of serious error are to be avoided. Attempts to assay the ergonovine content by difference between the results obtained for total activity and activity after its removal offer little hope of success. By way of illustration it is only necessary to recall that the highest accuracy ever claimed for the Cock's Comb or other biological methods for ergot involves an error of ten per cent. If the estimation of ergonovine depends upon two such assays, the *total* content of this substance in a sample of ergot may well fall within the limits of experimental error. Furthermore, the limited accuracy of methods commonly used in the past is suggested by the results of several workers who have obtained comparatively good agreement between the Cock's Comb method, which assays both ergonovine and alkaloids of the ergotoxine group, and the Broom-Clark method which assays only the latter group. In certain series of comparisons the Broom-Clark method, which theoretically should give lower results, showed in the majority of samples a higher value than did the Cock's Comb method. Consequently it would seem advisable to devise procedures which would assay the ergonovine fraction directly rather than by difference.

Another point which may be made concerns assays involving the uterine action of ergonovine. The possibility exists that alkaloids of the ergotoxine group, if present in appreciable amounts, even though having little effect themselves in producing uterine contractions, may inhibit the uterine stimulating action of ergonovine as suggested by the work of Thompson (10) and Swanson, Hargreaves and Chen (11) on the isolated rabbit uterus.

A further type of research which may well prove to be of value is that which played an important part in the isolation of ergonovine. In certain clinics the technique

has been developed for evaluation of oxytoxic drugs by the intrauterine balloon method on puerperal patients. Since the aim of ergot assay is the evaluation of the therapeutic effect of the drug, it should be helpful in establishing the validity of laboratory procedures to collaborate with clinical investigators whenever possible. Parallel assays of ergot or fractions of ergot on the human uterus and by laboratory methods should also offer additional information concerning the possible effects of one alkaloid or group of alkaloids on the action of another.

Finally, it is suggested that a step might be taken which would solve the problem of assay of galenical preparations, at least as far as the Pharmacopœia is concerned. The useful alkaloids are now available in pure form. They satisfy therapeutic requirements and the problem of assay and stability is much simpler for them than for the fluidextract of ergot. Ergonovine has undoubtedly replaced the galenical preparations to a considerable degree. A survey might supply useful information regarding the extent of such replacement. Is there any reason for retaining ergot and the fluidextract of ergot in the Pharmacopœia aside from the fact that a considerable proportion of the medical profession continue to use them because they are accustomed to do so? Probably this is sufficient reason for their retention, but it is believed that such a suggestion should at least be considered by the Revision Committee.

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Use of Sodium Pentobarbital for Repeated Anesthesia in the White Rat

*By V. Everett Kinsey**

In the present study the effects of giving sodium pentobarbital (Nembutal)[1] repeatedly to white rats will be compared with those observed previously (1, 2) for the rabbit and guinea pig.

The M. L. D. for rats for intraperitoneal injections of Nembutal has been reported as being 75 mg. per kilogram by Fitch and Tatum (3) and 120 mg. per kilogram by Carmichael (4) and Swanson and Shonle (5). Further disagreement is found in the reports concerning M. L. D. for male and female animals. None of these workers mentions a difference in response of the sexes. Barron (6) states that while the female is more susceptible to amytal than the male, there is no difference between the sexes so far as Nembutal is concerned. Holck and Kanan (7), on the other hand, state that the female rat is more sensitive than the male, as judged both by sleeping time and the M. L. D.; their assertion is confirmed by Moir (8) who found that the adult female rat is definitely less resistant than the corresponding male when the sleeping time was used as a criterion of sensitivity.

It would appear from the work of Carmichael (4), who showed that the M. L. D. in rats under nine months old was only 85–95 mg. per kilogram, as compared with 120 mg. per kilogram for rats older than this, that the comparison of M. L. D. sex differences, etc., must be made with animals of approximately the same age.

* Department of Physiological Chemistry, School of Medicine, University of Pittsburgh.

[1] Kindly supplied by the Abbott Laboratories, North Chicago, Ill.

The question of repeated anesthesia has been studied by Moir (8), who found that when Nembutal was given repeatedly, both the male and female rats developed a tolerance to this barbiturate as measured by the sleeping time. Stanton (9) has shown that only a minor degree of tolerance is developed to the maximal effects of Nembutal upon repeated anesthesia as indicated by the struggle response of rats one hour after giving injection.

MATERIALS AND METHODS

Albino rats (Wistar strain) about three to six months old, 125–250 Gm., were kept in large wire-bottom cages. They were fed Purina Dog Chow Checkers, supplemented with greens two or three times weekly. Water was given *ad lib.* Sodium pentobarbital was weighed out accurately and dissolved in sterile salt solution (0.9 per cent NaCl), containing 10 per cent ethyl alcohol, in concentrations of 6.5 mg. per milliliter. This solution was used in all of the experiments, excepting, of course, those which were designated specifically to test the influence of the alcohol; in the latter case the alcohol was omitted from the Nembutal solution in the control series. The injections were all made intraperitoneally after first wetting the area thoroughly with 70 per cent alcohol. The period during which the righting reflex was absent was used as the criterion of the sleeping time.

EXPERIMENTAL

The effect of the 10 per cent alcohol used in making up the solution of Nembutal was checked on both male and female rats. Using a standard dose of Nembutal of 31.3 mg. per kilogram, the average sleeping time of 7 male rats which were given the Nembutal solution containing 10 per cent alcohol (0.5 Gm. of alcohol per kilogram) was 71 minutes, compared to an average sleeping time of 79 minutes for 9 animals which were given the same dose of barbiturate, but no alcohol. An identical experiment was performed with female rats. Eight rats which were injected with 31.3 mg. per kilogram of Nembutal, containing 0.5 Gm. per kilogram of alcohol, slept 232 minutes compared to an average sleeping time of 231 minutes for 11 rats which were injected with the same amount of Nembutal, but no alcohol. Similar negative results were obtained when the same quantity of alcohol was included with other doses of the barbiturate which varied from 15.9 to 62.5 mg. per kilogram. The fact that the female rats slept so much longer than the male rats will be discussed later. The author (1, 2) had found that there was no appreciable evidence of synergism between ethyl alcohol and Nembutal in the guinea pig at similar dosage levels of alcohol, but that only one-tenth as large a quantity of alcohol was needed to more than double the sleeping time of rabbits when they were given 44 mg. per kilogram of Nembutal.

The standard dose of Nembutal of 31.3 mg. per kilogram was now given to 10 male rats daily and to 10 other male rats every other day for a period of 13 days. Two groups of 10 female rats each were treated in an identical manner. The average sleeping times for the 520 anesthesias are shown in Fig. 1.

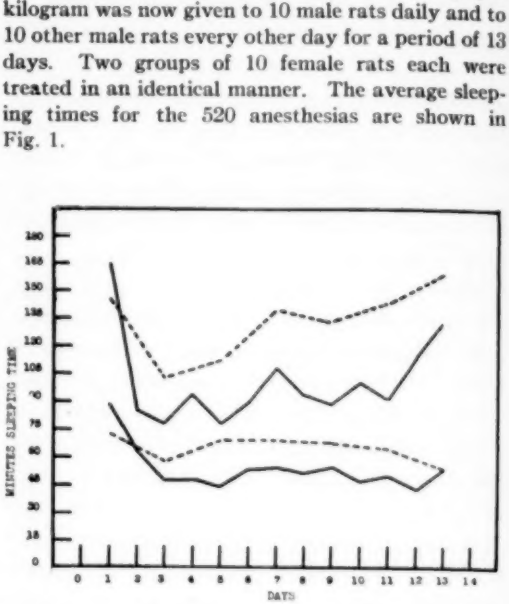

Fig. 1.—Showing the Average Sleeping Time of Rats Resulting from Intraperitoneal Injections of 31.3 mg. per Kg. of Nembutal. Top broken line—10 Female Rats Injected on Alternate Days. Top solid line—10 Female Rats Injected Daily. Lower broken line—10 Male Rats Injected on Alternate Days. Lower solid line—10 Male Rats Injected Daily.

From the two upper curves which represent the female rats, it may be seen that these rats sleep considerably longer than do the male rats, whether the injections are administered daily (solid line) or every other day. Certain it appears, too, that daily injections of Nembutal produce some tolerance to the drug in both sexes. Not so certain, however, is the repeated effect of the anesthesia when it is given every other day, since the sleeping times appear to be only slightly less, on the average, than they were originally. In this respect, likewise, the response of the rat is similar to that of the guinea pig, but different from that of the rabbit—which seems to require at least four days to lose the tolerance developed to repeated injections of Nembutal.

Because many investigators have been unable to find a difference in effect between the sexes to Nembutal, and because neither the guinea pig nor the rabbit seems to exhibit a difference in the response of the sexes, it was thought advisable to recheck the findings presented above. This was carried out on two additional groups of rats. Since it is possible that the diet may be responsible at least in part for the differences observed, one of the group (4 males and 5 females) was

kept on a synthetic diet[2] from the time of weaning until they were given the repeated anesthesias at the age of 14 weeks. The other group (6 males) was given the usual laboratory diet.

The average sleeping times for all of the males and females, injected daily and on alternate days, have been assembled in Table I. Notwithstanding the large variations in the individual sleeping times, it is evident that in all cases the female animals would sleep almost twice as long as the males when given the same dose of Nembutal. These observations confirm and extend the work of Moir (8) and Holck and Kanan (7) who had found previously that the female rat was more susceptible than the male.

Having investigated the response of the male and female rat to a particular dose of Nembutal it was decided to continue the study using increasingly larger doses. Thus it should be possible ultimately to find whether the female succumbed to a lesser dose than the male, as stated by Holck and Kanan (7), and perhaps to obtain some indication whether the M. L. D. is 75 mg. or 120 mg. per kilogram, as

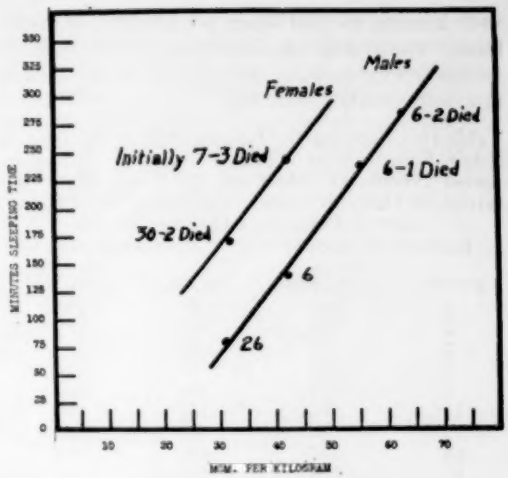

Fig. 2.—Showing the Sleeping Time Resulting from Increasing Doses of Nembutal.

Table I.—Showing the Length of Sleeping Time of Male and Female Adult Rats Following Repeated Intraperitoneal Injections of Nembutal (31.3 mg./Kg.)

Males					Females				
Number of Animals	Number of Injections per Animal	Average Sleeping Time, Min.	Range of Average Sleeping Time of Each Animal, Min.	Range of Individual Sleeping Time of Each Animal, Min.	Number of Animals	Number of Injections per Animal	Average Sleeping Time, Min.	Range of Average Sleeping Time of Each Animal, Min.	Range of Individual Sleeping Time of Each Animal, Min.
Injected Daily									
10	13	54.0	46–73	24–132[a]	10	13	101	80–124	44–201[a]
3	19	39.0	38–40	20–70					
4[b]	14	43.5	41–45	21–86					
Mean average 48.8 for 243 anesthesias					Mean average 101 for 130 anesthesias				
Injected Every Other Day									
10	7	63.8	61–71	38–102	10	7	133	106–164	61–210[a]
3	10	53.6	51–56	39–76	3[b]	7	91	77–101	31–197[a]
Mean average 61.5 for 100 anesthesias					Mean average 119 for 105 anesthesias				

[a] First day of injection.
[b] Synthetic diet (complete).

variously found by the workers cited in the first part of this paper.

The four doses of sodium pentobarbital selected for study were 31.3, 42, 55 and 62.5 mg. per kilogram. All of them were made up in 10 per cent alcoholic saline (0.9 per cent NaCl) solution. The results of these single injections appear in Fig. 2. There it may be seen that the sleeping time appears to increase directly with the dose, but, as in the case of guinea pigs, the dose must exceed a threshold value, which in the present case amounts to approximately 10 mg. per kilogram for the female and 20 mg. for the male. That the female is more susceptible than the male is further indicated by the fact that at a dose of 55 mg. per kilogram and above (with one exception at 62.5 mg. per kilogram) all of the female rats died, while only three out of twelve male rats died when given these doses. Finally, a M. L. D. value for male rats of somewhere near 75 mg. per kilogram is indicated (*cf.* Fitch and Tatum (3)) although too few animals were used at the higher dose levels to establish the M. L. D. precisely.

Moir (8) found that castrating rats increased their susceptibility to Nembutal as measured by sleeping time. The castrated animal, however, was not as susceptible as the female, whence Moir concludes that some factor other than the male gonad is responsible for male rats being less susceptible to the hypnotic effect of sodium pentobarbital than females. Incidently Barron (6) found that castrating rats increased their chance of dying when given minimal lethal doses of sodium amytal.

As Moir inferred, it would appear that some secretion of the testis decreases the susceptibility of rats to Nembutal. To establish this idea a little more fully, 20 female rats were divided into two equal-sized groups. The first group was given 0.1 mg. of testosterone propionate subcutaneously every day for ten days while no treatment was given the second group of rats. At the end of this time both groups

[2] Casein 18%, salt mixture 4%, butter fat 8%, cod liver oil 2%, cornstarch 68%, tiki-tiki extract from 250 Gm. of tiki-tiki, and 40 gamma per day of riboflavin.

were injected with 31.3 mg. per kilogram of Nembutal. The average sleeping times were 129 minutes for the treated rats and 230 minutes for the control rats, respectively (Table II).

Table II.—Showing the Length of Sleeping Time of Adult Female Rats Which Previous to Intraperitoneal Nembutal Injections (31.3 mg./Kg.) Received 10 Daily Subcutaneous Injections of Testosterone Propionate (1.0 mg. Total)

Received Testosterone		Controls	
Rat No.	Sleeping Time, Min.	Rat No.	Sleeping Time, Min.
1	109	6	284
2	124	7	202
3	29	8	235
4	132	9	274
5	234	10	234
11	126	14	239
12	89	15	121
13	137	18	258
16	178	19	220
20	Died		
	Average 129		Average 230

The outcome of this experiment suggests rather strongly that the testosterone does indeed confer some degree of resistance to Nembutal upon the animal receiving it. It should be noted, however, that the average sleeping time of the female rats given testosterone propionate still exceeds somewhat the average sleeping time (80 minutes) of a comparable series of male rats. This agrees with the result of Moir who found, it will be recalled, that the sleeping time of castrated rats was still not so long as that of comparable female rats.

While larger doses of testosterone propionate might still further lower the sleeping time of female rats, it seems that a hormone secreted by the ovaries might account for the fact that the female rats treated with testosterone are still somewhat more susceptible to Nembutal than are the males. To check this point 20 uniform young (150–215 Gm.) female rats were spayed, and after two weeks they were given 31.3 mg. per kilogram of Nembutal. Twenty other female rats similar in every way but unspayed, were used for controls. (The same rats were then used for work reported in Fig. 1.) Table III shows that the average sleeping times of the

Table III.—Showing the Effect of Spaying on the Sleeping Time of Rats When Anesthetized with 31.3 Mg. per Kilogram of Nembutal

No. of Rats	Average Sleeping Time, Min.	Range of Sleeping Time, Min.
	Controls	
20	156	110–210
	Spayed Rats	
20	98	70–137

spayed animals were reduced quite appreciably. It would be of interest to give testosterone propionate to spayed rats and determine whether all differences between the sleeping times of male and female rats as a result of Nembutal injections would then disappear.

In conclusion it should be emphasized that when animals are anesthetized with barbiturates, wide variations occur in the sleeping times of individual animals; and also that even when every known precaution is taken to control all variables, discrepancies may nevertheless occur. This should be borne in mind when interpreting any of the data reported above, obtained from numbers which may sometimes be statistically inadequate.

CONCLUSIONS

1. It has been shown that ethyl alcohol in concentrations of 0.5 Gm. per kilogram does not potentiate the action of varying doses of Nembutal in white rats as measured by the increased sleeping time.

2. As a result of administering 31.3 mg. per kilogram of Nembutal repeatedly to rats, the sleeping time decreases abruptly after the first dose when the injections are given at daily intervals. When the frequency of anesthesia is decreased to every other day, the decrease in sleeping time is not so noticeable.

3. Female rats sleep about twice as long as male rats when given either single or repeated injections of Nembutal.

4. Following the injection of 1 mg. of testosterone propionate subcutaneously into female rats, the average sleeping time resulting from a dose of 31.3 mg. per kilogram of Nembutal is reduced from 230 to 129 minutes.

5. Spayed rats seem to sleep only about two-thirds as long as unspayed controls.

The author wishes to express his gratitude to Miss Barbara Petty for her assistance throughout this work and to Dr. Charles F. Kutscher who made the study possible through his liberal support of this laboratory.

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"Any problem which is faced with complete honesty vanishes."—

NINA WILCOX PUTNAM.

The Alkaloids of the Chinese Drug Pai Pu*,†

By Henry M. Lee and K. K. Chen

It is well known that alkaloids occur in the species of *Stemona*, family *Stemonaceæ*. Table I is a résumé of the studies already recorded in literature. No information is as yet available concerning the chemical structure of these bases. Almost equally scanty is their pharmacological status. The only report dealing with the action of an alkaloid is that of Lobstein and Grumbach (5). These authors showed that their "stemonine" isolated from *S. tuberosa* caused paralysis of earthworms and frogs, reduced the chronaxie of frogs' muscle-nerve preparations and diminished the turtle's heart rate with increase in contraction soon followed by arrhythmia and depression.

Table I.—Alkaloids of *Stemona* Species

Species	Alkaloid	Empirical Formula	Melting Point, °C.	Optical Rotation	Investigated by
S. japonica	Stemonine	$C_{17}H_{23}O_4N$	151	$[\alpha]_D^{15}$ −113.8°	Suzuki (1)
	Stemonidine	$C_{19}H_{29}O_5N$	116	$[\alpha]_D^{15}$ −7.6°	
S. ovata	Isostemonidine	$C_{19}H_{31}O_5N$	137	$[\alpha]_D$ −48.9°	Suzuki (2)
S. sessilifolia	Hodorine	$C_{19}H_{31}O_5N$	...	.....	Furuya (3)
	Unnamed base	$C_{22}H_{33}O_4N$	86–87	$[\alpha]_D^{30}$ −47.1°	Schild (4)
S. tuberosa	Stemonine	$C_{22}H_{33}O_4N$	160	$[\alpha]$ +76.5°	Lobstein and Grumbach (5)
	Tuberostemonine	$C_{22}H_{33}O_4N$	86–88	$[\alpha]_D$ −25.4°	Suzuki (6, 7)

The Chinese drug Pai Pu is one of the *Stemona* species, although its exact botanical identity has not been established. Stuart (8) called it *S. tuberosa*, while the authors of "Botanical Nomenclature" (9) named it *S. sessilifolia*. The mere fact that Pai Pu gives rise to new alkaloids as shown in later sections is an indication that neither identification is correct. According to Pentsao Kang Mu (10), the root of this plant has been used in China as a cough remedy, an insecticide and in the treatment of pulmonary inflammation, scabies and insect bites. Recently Wang (11) reported that an alcoholic extract of Pai Pu was effective in killing lice.

EXPERIMENTAL

On account of the information furnished by Mr. Edward W. Miller that Pai Pu was attracting attention in modern medicine, we attempted to make a chemical investigation. Our material was obtained in the vicinity of Shanghai through the assistance of Mr. Miller. Briefly, two alkaloids were successfully isolated in crystalline form. They differed in both physical constants and chemical composition from the known alkaloids of *Stemona* species, as enumerated in Table I. One was water-soluble, melted at 138–138.5° C. and had the empirical formula $C_{21}H_{35}O_5N$; and the other was ether-soluble, melted at 105.5–106.5° C. and conformed to the empirical formula $C_{24}H_{37}O_4N$. The name sinostemonine is proposed for the higher melting compound, and paipunine for the other base. Certain pharmacological experiments were carried out with these new alkaloids.

I. CHEMICAL PART

The sample of Pai Pu, when assayed gravimetrically, was found to have a total alkaloidal content of 1.77 per cent. For isolation of the alkaloids, a quantity of 10 Kg. of the powdered root was percolated with ethyl alcohol until exhaustion. The alcohol of the extract was removed under diminished pressure and the residue was treated with dilute hydrochloric acid and filtered, to separate the resinous material. Crude alkaloids were then obtained by precipitation with ammonium hydroxide. Upon

Fig. 1.—Photomicrograph of Paipunine.

* From the Lilly Research Laboratories, Indianapolis, Indiana.

† Presented before the Scientific Section, A. PH. A., Richmond meeting, 1940.

filtering and drying, the mass was repeatedly extracted with ether and the combined extracts were subjected to evaporation. This gave rise to paipunine which could finally be purified by recrystallization from a mixture of isopropyl alcohol and water. The remainder of the alkaloidal mass after ether extraction was dissolved in hot water. On cooling and standing, sinostemonine crystallized out in abundance.

Paipunine melts at 105.5–106.5° C. and is levorotatory, its specific rotation in acetone being $[\alpha]_D^{25}$ −53.7°. It forms elongated prisms as shown in Fig. 1 and exhibits a blue fluorescence in alcohol or acetone—very strongly under ultraviolet rays. It is easily soluble in alcohol, acetone, ether and dilute acids, but slightly soluble in water. It reacts to form a precipitate with Mayer's and Wagner's re-

Fig. 2.—Photomicrograph of Sinostemonine.

agents and phosphomolybdic, phosphotungstic and picric acids. The results of combustion analyses conform to the empiric formula $C_{24}H_{37}O_4N$.

Analysis. Calculated for $C_{24}H_{37}O_4N$: C, 71.41; H, 9.25; N, 3.47. Found: C, 71.45; H, 9.24; N, 3.66; C, 71.46; H, 9.39; N, 3.64.

Sinostemonine melts at 138–138.5° C. and crystallizes in needles as illustrated in Fig. 2. It has in water a specific rotation of $[\alpha]_D^{25}$ −37°, and is soluble in dilute acids and hot water, but less soluble in ether, alcohol and acetone. Like paipunine, it has a blue fluorescence in organic solvents, particularly under ultraviolet rays. It precipitates Mayer's and Wagner's reagents and phosphomolybdic, phosphotungstic and tannic acids. When heated in vacuum at 100° C., it loses an average weight of 3.38 per cent, less than one molecule of water of crystallization. Analytical data suggest the simple formula $C_{21}H_{35}O_5N$.

Analysis. Calculated for $C_{21}H_{35}O_5N$: C, 65.92; H, 9.49; N, 3.66. Found: C, 65.88; H, 9.37; N, 3.51. C, 65.67; H, 9.20; N, 3.63.

It becomes obvious that the composition and physical constants of paipunine and sinostemonine are not in agreement with those of any known alkaloids of *Stemona* species. Indeed, evidence favors the contention that they are new alkaloids.

II. PHARMACOLOGIC PART

1. Paipunine.—For pharmacological experiments the alkaloid was dissolved in dilute hydrochloric acid. Comparatively higher concentrations such as a 1 per cent solution required a low p_H, 2 to 3, otherwise the base would settle out. Frogs injected in the lymph sac in the dose of 0.1 mg. per Gm., mice injected intravenously in the dose of 22 mg. per Kg. and rabbits injected intravenously in the dose of 20 mg. per Kg., all developed clonic convulsions. Mice jumped high in the air during violent convulsions, while rabbits and frogs took opisthotonos position. The action is apparently on the medulla for in frogs decerebration did not alter the symptomatology but destruction of the medulla abolished the convulsions.

Table II.—Toxicity of Paipunine and Sinostemonine in Mice by Intravenous Injection

Alkaloid	Dose, mg. per Kg.	Number Died / Number Used	LD_{50} ± Standard Error, mg. per Kg.
Paipunine	25–28	0/18	38.95 ± 2.09
	31	3/8	
	35	2/8	
	39	3/8	
	45	6/8	
	50	8/9	
'Sodium Amytal' immediately followed by Paipunine	56	1/8	81.62 ± 8.60
	75	4/7	
	100	5/8	
	133	7/8	
Sinostemonine	600	1/5	757.0 ± 53.5
	650	3/10	
	680	7/10	
	700	6/10	
	750	4/10	
	810	1/10	
	880	7/10	
	1000	5/5	

By intravenous injection of paipunine in mice, the median lethal dose (LD_{50} ± Standard Error) was determined to be 38.95 ± 2.09 mg. per Kg. as shown in Table II. In view of the fact that "Sodium Amytal" (Sodium Iso-amyl Ethyl Barbiturate, Lilly) is capable of detoxifying drugs which cause convulsions (12, 13, 14, 15, 16, 17, 18), such as cocaine, strychnine, picrotoxin, amidopyrine, dendrobine, coriamyrtin, tutin and harmine, it was tried here in mice in conjunction with paipunine. Indeed the results as contrasted in Table II strongly suggest the antagonism between paipunine and "Sodium Amytal." The latter appeared to detoxify slightly more than one LD_{50} of the former.

The action of paipunine on the frog's heart by perfusion into inferior *vena cava* is one of depression.

A concentration of 1:5000 caused a decrease in heart rate and amplitude of contraction, terminating in diastolic stoppage. After cardiac arrest, the ventricle responded by contraction to mechanical stimuli, and in most instances recovery took place when the medicated Ringer's solution was replaced by normal Ringer's solution. Vasoconstriction was observed in two out of three experiments in which the frog's legs were perfused with paipunine by the method of Laewen-Trendelenburg. There was no effect in the third animal.

In etherized cats intravenous injection of paipunine in the dose of 15–20 mg. (total) was followed sometimes by a rise, and other times by a fall, of carotid blood pressure. The results are difficult to interpret, but it is probable from the evidence in frog studies that the rise may be due to excessive vasoconstricting action as compared with cardiac depression, and *vice versa*. Respiration recorded with blood pressure showed depression followed by augmentation. It was not determined whether or not these changes were secondary to those of blood pressure.

Paipunine in the concentration of 1:50,000 inhibited the peristaltic movements of isolated rabbit's small intestines immersed in Locke's solution maintained at 38° C., as illustrated in Fig. 3. The action of barium chloride was abolished following paipunine. After ergotoxine had paralyzed the sympathetic endings, as evidenced by the epinephrine test, paipunine was still active. It is thus apparent that paipunine acts directly upon the smooth muscles of the intestine and not on the sympathetic nervous system.

The isolated guinea pig's uterus, on the other hand, responded to the application of paipunine by contractions in concentrations varying from 1:400,000 to 1:100,000. While the explanation for the deviation of action on the uterus from that on the intestine must be reserved for future exploration, it is possible that the smooth muscle of the guinea pig's uterus is not the same as that of the rabbit's intestine. Previous reports (19, 20, 21, 22, 23) published from this laboratory indicated that ephedrine, fritimine, retrorsine, dendrobine and seneciphylline also inhibited isolated intestines but stimulated the uterus.

2. Sinostemonine.—In all experiments, aqueous solutions of this alkaloid were used without acidification. The substance was much less toxic than paipunine. The median lethal dose (LD_{50} ± Standard Error) in mice by intravenous injection was found to be 757 ± 53.5 mg. per Kg. No systemic effects could be elicited even with relatively large doses. Its activity is therefore very low.

SUMMARY

1. The Chinese drug Pai Pu contains 1.77 per cent of total alkaloids.

2. Two new alkaloids have been isolated in crystalline form —paipunine, $C_{24}H_{37}O_4N$, and sinostemonine, $C_{21}H_{36}O_5N$.

3. Pharmacologically, paipunine is a convulsant, acting probably on the medulla. The median lethal dose in mice by intravenous injection is 38.95 ± 2.09 mg. per Kg. "Sodium Amytal" is capable of detoxifying more than one median lethal dose of paipunine in mice. Paipunine depresses the frog's heart in 1:5000 solution. It inhibits isolated rabbit's small intestines, but contracts the isolated guinea pig's uterus.

4. Sinostemonine has a median lethal dose of 757 ± 53.5 mg. per Kg. in mice when injected intravenously. It is practically inactive in other respects.

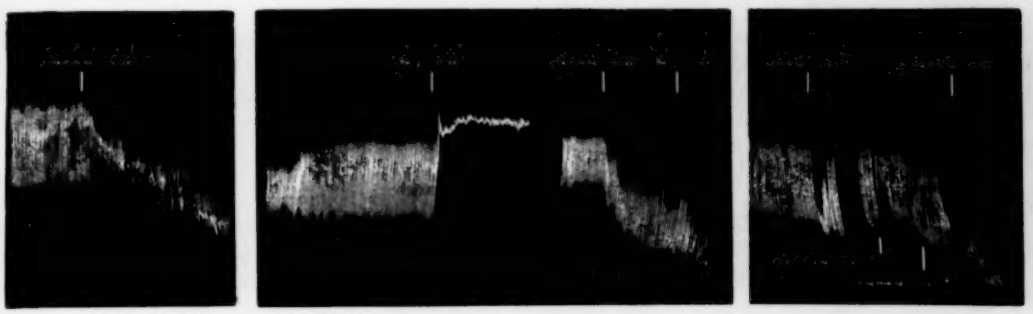

Fig. 3.—Illustration of the Effect of Paipunine on the Rabbit's Isolated Intestines.

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Tannic Acid, U. S. P.

II. Relationship between Glucose and Tannic Acid

By Clifton E. Miller and L. Wait Rising†*

It was claimed by Freda (1) in 1854 that tannic acid yielded on acid hydrolysis three molecules of gallic acid and one molecule of glucose, the glucose being a part of the tannic acid molecule. As a result of the study of the action of yeasts on tannic acid by Biddle (2) it was concluded that the glucose present in tannic acid is a free component and therefore an impurity.

EXPERIMENTAL

We endeavored to demonstrate the veracity of these seemingly conflicting theories by proving that glucose is not only a component part of tannic acid but an impurity as well. In order to do this, one per cent solutions of tannic acid were prepared and tested immediately by means of the Molisch reagent. A broad, distinct, violet band was formed. These one per cent solutions were then diluted to a strength of 1:1000. Using the Molisch reagent the violet band became narrower and fainter as the dilution increased. A solution freed of gallic acid also gave a positive test.

We therefore concluded that a sugar was present in the tannic acid samples as an impurity in varying amounts. As this reaction did not give any indication of the kind or amount of sugar, our attention was directed toward the possibility of preparing and isolating some derivative of the sugar in order to determine the kind and amount. If the amount of sugar was found to increase on hydrolysis it would prove possibly that the sugar existed in a combined form.

Osazones are usually crystalline in nature and can be easily purified by crystallization. The osazone prepared from the sugar in tannic acid after recrystallization from alcohol and pyridine was found to melt at 208° C. This led us to believe that the sugar was glucose and the fact that the sugar gave a negative Seliwanoff reaction was considered further proof of this.

The next step was the development of a method to determine the amount of osazone formed without the use of the tedious and unreliable gravimetric method. The unreliability of this method may be attributed to the solubility of the osazone in the large excess of phenylhydrazine acetate that is present in the mixture.

Knecht's method (3) of osazone estimation by means of titanium trichloride was modified by using methylthionine chloride instead of crystal scarlet. The solutions required were: standard titanium chloride solution prepared by mixing 50 cc. of commercial 20 per cent titanium chloride with 50 cc. of hydrochloric acid, specific gravity 1.175, and diluting to one liter with freshly distilled water; standard glucose solution containing 10 mg. per cc.; fifteenth molar methylthionine chloride solution; saturated sodium tartrate solution; and twenty-five hundredths per cent phenylhydrazine in glacial acetic acid.

The titanium chloride solution was standardized both in terms of milligrams of glucose per cc. of solution which is hereafter called the glucose factor and cc. of methylthionine chloride solution. The latter standardization was made at a temperature of 70° C. in the presence of hydrochloric acid. It is advisable to determine the glucose factor for each lot of commercial titanium chloride for accurate results. The end-point of the titration is the formation of a permanent blue color, which is advantageously seen by directing a beam of light from beneath the flask.

For the hydrolysis, a 20 per cent solution of each sample in approximately tenth-normal sulfuric acid was heated continuously on a steam bath. In order

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to determine the amount of glucose formed as a result of the acid hydrolysis, samples of each solution were taken for analysis prior to heating. During the heating procedure samples were withdrawn at the end of each five-hour period, cooled to room temperature and the glucose determined in the following manner.

Two cc., representing 40 mg. of tannic acid, of the hydrolysis mixture, two cc. of saturated sodium tartrate solution and two cc. of the phenylhydrazine solution were placed in a test-tube and heated in a boiling water bath for ten minutes. The hot solution was transferred to a small flat-bottom flask and a known excess of titanium chloride solution added. The contents of the flask were boiled over a free flame for two minutes. The mixture in the flask which had a chocolate color was acidified with seven and a half cc. of hydrochloric acid, specific gravity 1.175, and the hot solution titrated with methylthionine chloride to determine the excess titanium chloride. Having found the amount of titanium chloride used, the glucose factor was multiplied by the amount of titanium chloride to obtain the amount of glucose.

We were able to calculate the quantity of glucose formed during the hydrolysis by subtracting the amount found previous to the heating from that found at the completion of the hydrolysis. Constant results at the end of two five-hour periods determined the end-point of hydrolysis. The heating was continued beyond this point on several samples and it was found that there was no change in the results. The amount of sugar is designated in terms of milligrams per 40 mg. of tannic acid in Table I, and represents the total free glucose at each five-hour interval.

Table I.—Total Free Glucose at Each Five-Hour Period

Hours of Heating	Sample 1	2	3	4	5	6	7	8	9	10	11	12
0	5.15	2.97	3.31	2.87	2.28	4.65	3.06	3.55	2.33	2.62	2.81	2.60
5	4.51	2.77	4.75	5.05	3.96	5.35	2.89	2.40	2.71	2.96	2.57	3.30
10	4.35	3.86	3.26	5.35	3.66	6.53	3.26	3.87	2.89	2.89	2.64	3.01
15	6.10	3.36	4.31	5.25	3.46	5.25	3.31	3.50	3.62	2.52	3.01	3.87
20	5.84	4.16	4.73	5.38	4.31	5.14	3.38	3.87	3.38	2.89	3.13	3.74
25	6.10	3.17	5.09	4.45	3.07	4.95	3.84	4.24	3.62	3.87	3.62	3.62
30	5.19	3.36	3.75	5.44	3.46	4.54	4.65	3.85	3.74	3.99	3.74	3.74
35	5.74	4.36	5.22	5.80	3.82	6.63	4.48	4.36	3.71	3.74	3.87	3.71
40	6.34	3.06	4.12	4.85	4.45	4.95	4.65	3.87	3.92	3.74	4.11	4.68
45	5.94	4.95	5.34	5.28	4.12	5.74	5.34	4.36	4.25		5.34	4.71
50	5.74	4.65	5.07	4.85	4.26	5.74	5.09	5.09	4.36		4.48	4.82
55	5.94	4.35	6.19	6.23	3.96		5.95	5.09	4.94		5.21	4.71
60	6.10	4.35	3.95	5.84	4.26		5.95		4.65		5.58	5.09
65	6.83		4.85	6.15	4.61				4.72		5.29	4.11
70	6.34		5.34	6.47	6.04				5.21		5.46	5.44
75	7.92		7.17	7.03	6.04				5.46		5.70	5.56
80	6.63		5.83	6.63					5.46		5.28	6.44
85	6.75		5.83	6.53							5.95	5.95
90	8.51			8.01							5.95	5.70
95	7.62			7.33								5.58
100	8.21			7.33								6.32
105	8.21											6.05
110												6.05

Table I shows the amount of sugar formed as a result of the hydrolysis and the amount of sugar present as an impurity.

Table II.—Sugar Formed by Hydrolysis and as an Impurity

	Sample 1	2	3	4	5	6	7	8	9	10	11	12
Total sugar, %	20.40	10.80	14.50	19.20	15.00	14.20	14.30	12.70	13.60	9.30	14.90	15.10
Sugar as impurity, %	12.80	7.10	8.20	7.50	5.60	11.60	7.60	8.80	5.80	6.30	7.00	6.70
Sugar formed, %	7.60	3.70	6.30	11.70	9.40	2.60	6.70	3.90	7.80	3.00	7.90	8.40

A possible explanation of the vacillation in the sugar formation is that glucose and gallic acid in the presence of an acid may unite to form an unstable compound which will break down to give sudden maximum concentrations of glucose. It may also show that in some samples there is relatively more gallic acid present as an impurity.

CONCLUSIONS

1. The acid hydrolysis of Tannic Acid, U. S. P. has been studied quantitatively with respect to its glucose content.

2. A quantitative method for the determination of glucose in Tannic Acid has been developed.

3. It is evident that the glucose is present in variable amounts as an impurity and for this reason different samples of the same weight of Tannic Acid would not necessarily yield the same amount of glucose on hydrolysis.

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Tannic Acid, U. S. P.

III. Hydrogen-Ion Studies of Tannic Acid Solutions

By Clifton E. Miller and L. Wait Rising†*

It is well known that freshly prepared solutions of tannic acid in water, irrespective of concentration, show a marked change in color, formation of bacterial growth, formation of an odor and, in some cases, deposition of a residue when kept over a prolonged period of time. These changes appear to be the result of decomposition. Engels (1) and Fantus (2) introduced various stabilizing agents in an attempt to prevent these changes. Therefore it was desired to measure the degree of decomposition of the stabilized and unstabilized solutions if possible. One way to do this on a comparative basis is to measure the resultant changes in hydrogen-ion concentration.

Surprisingly little work has been done on the hydrogen-ion value of tannic acid solutions. Johnson (3) in his survey of hydrogen-ion studies makes no mention of tannic acid-p_H relationship; Glasstone (4) proposed the limits of p_H in which it was possible to obtain a violet color by the addition of ferrous tartrate solution to solutions of tannic acid. This was another reason why the study seemed worth while.

EXPERIMENTAL

As tannic acid is water-soluble and does ionize, it seemed of interest to make a relative study of the hydrogen-ion concentration of freshly prepared solutions and stabilized solutions of tannic acid over varying intervals of time.

Therefore freshly prepared one per cent aqueous solutions were tested at varying intervals for a period of 96 hours. The hydrogen-ion value was determined using a saturated calomel-quinhydron, electrode. All readings were made at 250° C.

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Table I.—p_H Values of Freshly Prepared Tannic Acid Solutions

Sample	Hours 0	24	72	96
1	2.5	2.5	2.6	2.8
2	2.6	3.1	2.9	3.0
3	2.6	2.8	3.1	3.2
4	2.4	2.6	2.8	3.1
5	2.8	2.8	3.0	3.4
6	2.7	2.7	3.3	3.2
7	2.8	2.7	3.2	3.1
8	3.8	3.4	3.6	3.5
9	3.6	2.9	3.6	3.0
10	3.6	2.9	3.2	3.2
11	3.4	3.2	3.1	3.4
12	3.2	2.9	3.0	3.4

It is quite evident that aqueous solutions of tannic acid are unstable on standing and their internal constitution is not only constantly changing but generally toward the alkaline side.

As mentioned earlier, Fantus brought forth a formula for the stabilized solutions of tannic acid in which he proposed the use of stabilizers such as salicylic acid and sodium chloride. Solutions were prepared using the formula suggested by Fantus which is given herewith.

Potassium chloride	0.42 Gm.
Calcium chloride	0.84 Gm.
Salicylic acid	1.00 Gm.
Sodium chloride	10.50 Gm.
Tannic acid	100.00 Gm.
Distilled water *q. s.*	1000.00 Gm.

The p_H of the solutions was determined upon preparation and at two-month intervals over a period of 12 months. There was a general darkening of the solutions as time progressed. Generally the acidity decreased in proportion to the increase in time. A sediment was noted in the bottoms of the containers which were kept under ordinary laboratory conditions.

Table II.—p_H Values Using Fantus Formula at Two-Month Intervals

Sample	Months 0	2	4	6	8	10	Av.
1	3.0	2.7	2.4	2.5	2.6	2.6	2.6
2	2.9	3.3	2.8	2.9	3.2	3.4	3.1
3	3.1	4.3	4.0	4.4	4.2	4.1	4.0
4	2.8	4.0	3.2	3.6	3.2	3.2	3.3
5	3.1	3.9	3.8	2.2	4.0	3.9	3.5
6	2.7	4.4	3.7	4.0	3.8	4.0	3.8
7	2.5	4.0	3.6	4.2	3.5	3.9	3.6
9	2.9	4.2	3.6	4.1	3.6	4.1	3.7
10	2.9	4.3	3.7	4.2	4.2	3.7	3.9
11	2.9	4.5	3.6	3.7	3.8	3.8	3.7
12	2.9	4.2	3.7	3.9	3.5	3.7	3.7

Since Sample No. 8 presented difficulties in getting a clear solution using the formula of Fantus no results are given.

Solutions were also prepared using Engels' formula, which is given herewith.

Alkyl or aryl *p*-hydroxy benzoate	0.1000 Gm.
Sodium bisulfite	2.5000 Gm.
Tannic acid	50.0000 Gm.
Distilled water *q. s.*	1000.0000 Gm.

Four sets of solutions were made up according to the above formula using methyl, ethyl, propyl and benzyl *p*-hydroxy benzoates as stabilizers. No similarity in color could be detected and after standing for a period of 10 months the samples had noticeably darkened in color. p_H determinations were made at two-month intervals.

Table III.—p_H Values, Methyl Para Hydroxy Benzoate as Stabilizer

Sample	Months 0	2	4	6	8	10	Av.
1	5.2	3.6	4.8	5.0	5.0	2.6	4.4
2	5.7	3.6	5.3	5.1	5.0	4.5	4.9
3	5.5	3.5	5.4	5.4	5.1	4.6	4.9
4	5.6	3.1	5.7	5.3	4.9	4.3	4.8
5	6.1	3.4	5.6	5.5	5.0	3.5	4.8
6	5.9	3.3	5.8	5.7	5.3	4.5	5.1
7	6.0	2.8	5.7	5.5	5.1	4.5	4.9
8	6.1	3.8	6.0	6.0	5.4	4.2	5.2
9	6.1	3.3	5.6	5.5	5.2	4.6	5.1
10	6.1	3.4	5.5	5.4	5.0	4.5	5.0
11	5.9	3.3	5.7	5.4	5.0	4.5	5.0
12	5.9	2.8	6.0	5.5	5.3	4.5	5.0

Table IV.—p_H Values, Ethyl Para Hydroxy Benzoate as Stabilizer

Sample	Months 0	2	4	6	8	10	Av.
1	5.3	3.8	6.1	5.7	5.5	4.6	5.3
2	6.1	4.0	5.7	5.6	5.1	4.5	5.2
3	5.9	4.7	6.2	5.6	5.5	4.6	5.4
4	5.9	4.0	5.9	5.4	5.1	2.8	4.8
5	5.8	4.2	5.9	5.5	5.2	4.2	5.1
6	5.5	4.1	6.1	5.6	5.3	4.3	5.1
7	5.6	4.3	6.2	5.5	5.3	2.8	4.9
8	7.9	5.2	6.7	6.3	5.9	4.5	5.9
9	6.3	4.4	6.2	5.7	4.8	3.7	5.2
10	5.9	4.5	5.9	5.5	5.2	4.1	5.2
11	6.0	4.4	6.6	5.4	5.2	4.3	5.3
12	6.2	4.1	6.1	5.9	5.3	4.2	5.3

Table V.—p_H Values, Propyl Para Hydroxy Benzoate as Stabilizer

Sample	Months 0	2	4	6	8	10	Av.
1	6.0	3.0	6.5	5.6	5.2	4.4	5.1
2	6.3	3.2	6.2	5.4	5.2	3.2	4.9
3	6.5	3.8	6.5	5.7	3.9	3.3	4.9
4	6.0	3.5	6.0	5.3	4.9	4.3	5.0
5	6.5	3.7	5.9	5.6	5.1	3.3	5.0
6	6.2	3.8	5.0	5.8	6.0	4.5	5.2
7	6.1	3.9	6.2	5.6	2.9	2.2	4.5
8	6.7	4.7	6.6	6.2	5.3	3.4	5.5
9	6.1	3.9	6.3	5.7	5.1	3.2	5.0
10	6.6	3.8	6.2	5.6	5.0	4.2	5.2
11	6.9	3.9	6.5	5.6	5.0	4.5	4.9
12	6.3	3.6	6.3	5.7	5.3	4.5	4.8

Table VI.—p_H Values, Benzyl Para Hydroxy Benzoate as Stabilizer

Sample	Months 0	2	4	6	8	10	Av.
1	6.3	2.4	6.3	5.7	4.8	3.4	4.8
2	6.2	2.9	5.8	5.7	5.1	4.3	5.0
3	6.5	3.2	6.2	5.7	5.4	4.3	5.2
4	5.9	2.5	5.8	5.0	2.9	2.4	4.1
5	6.1	2.9	6.1	5.6	4.9	4.3	5.0
6	5.7	3.0	6.8	5.7	4.8	4.4	5.1
7	6.7	3.5	6.3	5.8	5.0	4.4	5.3
8	6.5	3.8	6.8	6.3	5.7	4.9	5.7
9	6.4	3.7	6.1	5.4	4.4	2.7	4.8
10	5.6	2.9	6.0	5.7	5.4	4.6	5.0
11	5.9	3.9	5.9	5.7	5.2	4.8	5.2
12	5.6	4.2	6.1	3.9	4.3	3.8	4.6

CONCLUSIONS

1. A study of the rate of change of hydrogen-ion concentration of tannic acid solutions has been made.

2. Ethyl and propyl para hydroxy benzoates exhibited a stronger stabilizing power on tannic acid than the corresponding methyl and benzyl esters.

3. We believe that the recommended stabilizing agents do not produce the desired effect with respect to hydrogen-ion concentration.

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Adaptation of Assay Methods for Some N. F. Ointments. Ointment of Zinc Stearate*

By Wm. B. Baker and D. I. Kutzly

Many ointments in the N. F. VI do not have assay methods given for the determination of their active constituents. In the case of a number of these ointments, it has been found (1) that suitable assay procedures for the quantitative determination of certain medicinal ingredients are possible by adaptation of the assay methods that already exist for certain of the drugs and chemicals contained in the formulas.

OINTMENT OF ZINC STEARATE

The assay method for Zinc Stearate, U. S. P. XI, may be successfully adapted for use in the assay of Ointment of Zinc Stearate, N. F. VI, as shown by the satisfactory results reported in this article.

"Zinc Stearate is a compound of zinc with variable proportions of stearic acid and palmitic acid, corresponding to not less than 13 per cent and not more than 15.5 per cent of ZnO" (2). Therefore, the criterion of

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the assay for purity of Zinc Stearate and the content of Zinc Stearate in Ointment of Zinc Stearate depends upon the zinc oxide content.

EXPERIMENTAL

A sample of Ointment of Zinc Stearate was prepared according to the directions given in the N. F. VI. The Zinc Stearate used in the ointment was examined for purity prior to its incorporation into the ointment sample.

Table I.—Results of Assay of Zinc Stearate (U. S. P. XI Method)

Wt. of Sample, Gm.	$N/10$ H_2SO_4 Added, Cc.	$N/10$ NaOH Required, Cc.	Excess Acid Consumed, Cc.	ZnO, %
1.0051	50.35	17.14+	33.21−	13.44
1.0065	50.35	17.02+	33.33−	13.47
1.0043	50.35	16.91−	33.44+	13.55
1.0028	50.35	17.26−	33.09+	13.43
			Average	13.47

Table II.—Results of Assay of Ointment of Zinc Stearate for Zinc Oxide

Wt. of Sample, Gm.	$N/10$ H_2SO_4 Added, Cc.	$N/10$ NaOH Required, Cc.	Excess Acid Consumed, Cc.	Gm. of ZnO per 100 Gm. Ointment of Zinc Stearate
1.0010	50.35	38.83−	11.52+	4.68
0.9998	50.35	38.48−	11.87+	4.83
1.0053	50.35	38.59+	11.76−	4.76
1.0007	50.35	38.83−	11.52+	4.69
			Average	4.7396

Calculated percentage of zinc oxide in sample (35×13.47) = 4.7145
Average percentage of zinc oxide found in sample = 4.7396
Difference = 0.0251
Percentage error ($0.0251 \div 4.7396$) $\times$ 100 = 0.53

Assay Procedure for Zinc Oxide.—Place about 1 Gm. of the ointment, accurately weighed, in a tared crucible of about 30-cc. capacity. Heat the crucible and contents *gently* over a Bunsen flame until the ointment is liquefied. Gradually increase the temperature and ignite to eliminate the ointment base. Digest the residue remaining in the crucible after ignition with 50 cc. of tenth-normal sulfuric acid until solution is complete. Then titrate the excess of tenth-normal sulfuric acid with tenth-normal sodium hydroxide, using methyl red T.S. as the indicator. Each cc. of tenth-normal sulfuric acid is equivalent to 0.004069 Gm. of ZnO.

DISCUSSION

The base of Ointment of Zinc Stearate is composed of liquid petrolatum and white petrolatum. These ingredients are carbonized (along with the stearic acid and palmitic acid portions of the zinc stearate) upon ignition, leaving a residue of zinc oxide, which is determined titrimetrically.

The procedure for the determination of the active ingredient is simple, and accurate results are obtained.

RECOMMENDATION

It is recommended that the method described above for the determination of zinc stearate in Ointment of Zinc Stearate be adopted for admission to the National Formulary, and that the following standard be prescribed for the Ointment: Ointment of Zinc Stearate contains not less than 4.2 per cent and not more than 5.8 per cent of ZnO.

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Postage stamps have been issued by Italy in honor of the following: Leonardo da Vinci, painter, sculptor, architect and scientist; Alessandro Volta, electrophysicist; Luigi Galvani, physicist and anatomist.

Marihuana Investigations. II

The Effect of Variety, Maturity, Fertilizer Treatment and Sex on the Intensity of Response to the Beam Tests

By John R. Matchett, Joseph Levine* and Louis Benjamin,* B. B. Robinson† and O. A. Pope†*

INTRODUCTION

In a previous communication (1) it has been pointed out that (1) individual Cannabis plants, though of the same agronomic variety and grown side by side, vary widely in capacity to respond to the acid and alkaline Beam tests; (2) the substance responsive to the tests, when detectable at all, is always found in the tops and is generally found in all parts of the plant except pith, lower stalk and roots; (3) many plants respond to the tests when as small as three inches tall; (4) response continued throughout the life of the plant; and (5) male and female plants respond alike to the tests.

With a view to continuing the study of these and related phenomena the research here reported was initiated. Its objectives were to study (1) variation in response to the tests among different agronomic varieties; (2) the effect of fertilizer treatments on response to the tests; (3) variation in response with age of plants; and (4) difference in responses on the part of male and female plants.

Since the completion of the present research a pure substance, known as cannabidiol, has been isolated from Cannabis resin (2). This compound has been found to respond intensely to the alkaline Beam test. Cannabidiol accounted for a considerable percentage of the resin from which it was prepared and it is not unreasonable to assume that it is the chemical individual to which the test is principally due. It would appear that intensity of response to the alkaline Beam test constitutes a valid criterion of differences in chemical makeup of resins of different origin despite the fact that the cannabidiol has been found physiologically inactive in dogs.

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The acid Beam test, while by no means so firmly founded, chemically, must also be considered a measure of some quality of the resin and the test results described below are accordingly considered worthy of record though of lesser interest than those obtained employing the alkaline Beam test. There is evidence that intensity of response to the acid test is conditioned by the method of testing. The results recorded were obtained, of course, following a strictly uniform procedure.

EXPERIMENTAL

Material and Methods Employed.—On April 30, 1938, a plot of Cannabis was planted on the lowlands of the Department of Agriculture Experimental Farm in Arlington, Virginia, which was adjacent to the plot planted for the 1937 investigations. The plot consisted of twelve rows, three and one-quarter feet apart and one hundred and thirty feet long, the inner ten rows being divided into ten parts each, thus forming one hundred sections, ninety-six of which were utilized for planting the six agronomic varieties studied. Each variety was planted in sixteen randomized plots.

The outer rows and four remaining sections were not concerned in this report except that they assured identical conditions of sunshine and crowding to the remainder of the plot.

Fertilizer was applied to the various experimental plots. Nitrogen was applied in the form of sodium nitrate at the rate of 100 pounds per acre, 18% superphosphate at the rate of 200 pounds per acre and muriate of potash at the rate of 100 pounds per acre. This was applied directly in the row at the time of planting. By using each of these three fertilizers by itself, by combining them in couplets, by using them all together, there weres even different treatments provided for each of the six varieties. Adding to this a control plot for each of the six varieties, there results a total of eight different treatments, or 48 combinations of treatment *versus* variety. In view of the fact that each of these plots was planted in duplicate, 96 supervised plots were set out.

The varieties planted were as follows: Seed from Spantov province, Roumania, called Roumanian No. 1; seed from Canad province, Roumania, called Roumanian No. 2; seed from Cluj province, Roumania, called Roumanian No. 3; seed from Manchuria, called Manchurian; seed from China, called Chinese; and seed from Italy, called Italian.

Early growth was slow, presumably due to drought. Some male plants began flowering when only eighteen inches tall, but all continued to grow, as the season progressed, to an average height of nine feet.

By June 10th male flowers began to appear among the Roumanian varieties and by July 28th the sex

of all plants was apparent. By September the males were fast being denuded.

The growth types were as follows:

A. Plants short in height and very early in maturing. The internodes were shorter than the other types and the lateral branches shorter and gave the appearance of being much more compact. Roumanian No. 1 and No. 3 represented this type.

B. Plants medium tall, open branching heads and medium in time of maturing. Represented by Roumanian No. 2.

C. Plants tallest in growth type, retaining very few branches but of open branching growth type and medium in time of maturing. The most favorable type for the production of fiber. Represented by the Italian variety.

D. Plants medium tall with open branching type of growth but late in maturing. The *D* type is similar in many ways to the *B* type, except that the *D* is later in maturing than the *B* type represented by the Manchurian and Chinese varieties. The Chinese variety had very broad leaflets which could be seen and distinguished at a distance from the other varieties. The Manchurian differed from the Chinese in this respect, that its leaflets were narrower.

On three occasions during the growth of the plants, portions of the tops were cut from each of three plants in each of the ninety-six plots, making a total of two hundred and eighty-eight samples tested on each occasion. An effort was made to sample one male and two females on each occasion, but in the two earlier tests it was frequently not possible to distinguish the sex. Each plant, however, was tagged and its sex ultimately determined. Only a half dozen plants, thus tagged, died during the testing period. The samples gathered were dried in the laboratory air.

Date	Days Old	Average Height, cm.	Stage Growth	Gathered Males	Gathered Females	Drying Period	Temp. of Drying, °C.
6-18-38	49	60–100	Few flowers	133	155	4 days	30
6-30-38	61	120–200	Flowering	121	167	11 days	25
7-29-38	90	240	Full flower	96	192	7 days	25

Above are tabulated pertinent data regarding the plants on each of the three test occasions:

The test procedure employed was essentially that described in a previous report (1). The uniformity of the samples permitted the procedure to be standardized as follows:

1. Cover 1/2 Gm. of crushed sample with 15 cc. of pure ethyl acetate and allow to extract 1 minute or more.

2. Add 1/2 Gm. of Norit (activated carbon) and stir.

3. Filter through a dense paper and place 5 cc. of filtrate in each of two white porcelain dishes.

4. Evaporate the solvent on a steam bath before a fan.

5. *Alkaline Test.*—To the residue in one dish add 5 drops of Beam's alkaline reagent (2% alcoholic potassium hydroxide) and observe any purple or violet color which appears within thirty minutes.

6. *Acid Test.*—To the residue in the second porcelain dish add 1 cc. of Beam's acid reagent (saturated alcoholic HCl) and observe any cherry-red color which develops at once.

The plants were classified in six categories based on intensity of response to the tests. Those referred to as "0" were negative; those referred to as "1" showed a trace of color; and the other classes showed color intensity increasing in the indicated order. It may be stated that tests listed as "2," "3," "4" or "5" were clearly positive. Those listed as "1" could scarcely be considered adequate for identification, although a definite trace of color was produced in each case.

Tests were made in the numerical order of test plots, which numbers were random with respect to the various varieties and fertilizer treatments. In this way those making the tests were not aware at any time which variety or treatment was being tested. The data recorded were assembled after all chemical work had been completed.

Table III presents the results of an "Analysis of Variance" of all the alkaline and acid test data showing the effect thereon of each of a number of variables and combinations of variables. Under the heading "required" appear the numbers which must be derivable from the data variations if the probability that the indicated effect of the corresponding variable is not due to chance is to be 19 to 1 or 99 to 1 as the case may be. In the column headed "Found" are recorded, opposite the pertinent variable, the numbers actually derived where these were found to be larger than the required numbers. Where no number is recorded the number derived was numerically smaller than the required value and the effect of the variable in question is not to be considered statistically significant.

RESULTS

ALKALINE TEST

The data obtained from the testing are assembled in Table I. Each of the individual numbers recorded is the sum or total of the "test intensity numbers" of the three individual plants tested from each of the 96 plots. The data for each of the three test occasions are separately grouped. The two numbers under each "Variety—Fertilizer Treatment" heading record the totals from duplicate plots. The sub-totals are the sums of all the individual totals occurring in the columns. The other totals are self-explanatory.

Effect of Variety.—Attention is invited to the striking difference in test response among the various varieties as reflected in the columns headed "Variety Sub-total" and "Variety Grand Total." The three Roumanian varieties are thus shown to re-

spond intensely to the test while the Chinese and Manchurian varieties respond very feebly. These differences are statistically significant. All of the plants which tested negatively occurred in the latter two varieties and the great majority of their positive responses were categorized "1" and "2." The Italian variety is intermediate in intensity of test response but more nearly resembles the Roumanians than the others. Of chief concern is the demonstration that samples of Manchurian or Chinese Cannabis may be encountered which cannot be identified by the alkaline Beam test as at present carried out. A considerable amount of the hemp now growing in this country is originally of Manchurian or Chinese origin.

Effect of Age.—The sum of the variety sub-totals for each of the three samplings as recorded in Table I indicate the differences that appeared in test response with advancing age of the plants. The most intense colors were obtained on the second occasion when most of the plants were flowering. The difference between these figures and those obtained on the first occasion are statistically significant, but the small diminution in test totals recorded between the second and third test occasions may be due to chance variation. It is noteworthy that the number of negative ("0") tests did not vary considerably among any of the three test occasions. The increasing intensity was accompanied by a corresponding decrease in the feebly positive categories "1" and "2." Since the period covered by the tests began before inflorescence and continued through the beginning of decadence of the males it would appear representative of the whole growth period. It is indicated that intensity of response to the test increases as the plants mature though previous tests have elicited strong responses in very young plants.

Effect of Fertilizer.—The sub-totals and grand totals of the vertical columns in Table I illustrate the differences in test response determined by the fertilizer treatments of the various plots. The figures do not vary considerably and it is inferred from the statistical analysis that under the conditions studied the fertilizer treatments used do not demonstrably affect the capacity of the plants to elaborate the substance, or substances, responsive to the test.

Effect of Sex.—Variation in test response due to sexual characteristics of the plants is shown in the first half of Table IV where the figures given in the several columns indicate the percentage of the total plants of each variety and sex which were categorized under each of the "test intensity number" classifications. If all plants of each sex be considered, irrespective of variety, no difference is to be found in test response between male and female plants. It is of interest to note, however, that if

Table I.—Tabulation of Alkaline "Test Intensity Totals." The Individual Entries Are "Test Intensity Totals" for the Three Plants Sampled from Each of the 96 Plots on the Date Indicated. The Totals in the Column Headed "Variety Grand Totals" Are the Sums of the Individual Variety Totals for the Three Test Occasions. The Indicated Fertilizers Are "N"—Nitrogen, "P"—Phosphorus, "K"—Potassium

Variety	Fertilizer Treatment: None		N		P		K		NP		NK		PK		NPK		Variety Sub-totals	Variety Grand Totals
First Sampling—June 18, 1938																		
Chinese	0	2	1	1	3	2	0	2	4	3	1	1	4	3	0	2	29	
Manchurian	3	1	0	3	1	6	3	3	2	2	9	2	2	2	5	4	48	
Italian	7	7	6	9	6	9	8	5	3	7	8	7	5	9	7	7	110	
Roumanian No. 1	9	9	9	10	5	9	9	9	8	10	4	12	9	9	7	9	137	
Roumanian No. 2	10	9	12	10	6	9	9	10	9	9	8	9	9	8	9	9	145	
Roumanian No. 3	6	9	12	9	9	9	9	9	9	9	8	9	8	9	8	9	141	
Fertilizer Sub-totals	72		82		74		76		75		78		77		76			
Age Total																	610	
Second Sampling—June 30, 1938																		
Chinese	3	6	4	3	2	5	6	1	3	2	3	1	3	1	4	1	48	
Manchurian	2	1	4	0	5	1	5	1	4	1	3	3	4	2	3	4	43	
Italian	10	9	10	9	10	10	11	8	9	9	10	10	9	10	10	9	153	
Roumanian No. 1	12	9	12	9	7	9	9	10	9	8	9	9	12	10	12	10	156	
Roumanian No. 2	10	12	8	11	9	10	12	10	10	8	11	10	10	10	8	9	158	
Roumanian No. 3	9	7	9	9	8	7	9	9	9	9	11	10	9	9	10	9	143	
Fertilizer Sub-totals	90		88		83		91		81		90		89		89			
Age Total																	701	
Third Sampling—July 29, 1938																		
Chinese	3	2	2	1	2	1	7	1	2	4	4	1	0	2	1	0	33	110
Manchurian	2	2	5	4	1	2	3	3	1	3	3	0	3	3	1	3	39	130
Italian	8	9	9	9	9	9	10	8	9	8	9	9	7	9	9	9	140	403
Roumanian No. 1	9	10	10	9	10	10	10	9	10	8	10	12	8	10	8	7	150	443
Roumanian No. 2	13	9	9	8	11	10	11	9	11	9	11	9	11	10	12	12	165	468
Roumanian No. 3	10	12	11	9	10	10	10	10	13	10	11	9	9	9	11	12	166	450
Fertilizer Sub-totals	89		86		85		91		88		88		81		85			
Age Total																	693	
GRAND TOTALS	251		256		242		258		244		256		247		250			

each variety be considered separately, three show slightly greater "test response" means among the males, and the other three show slightly greater "test response" means among the females. It must be concluded that difference in test response between male and female plants, if it exists at all, is of no considerable consequence. No inference may be drawn from these results regarding the relative physiological potencies of male and female plants.

ACID TEST

Table II and the second halves of Tables III and IV record the acid test data. The significance of the various numbers in the tables is similar to that referred to above for the alkaline test data.

It is plain that no very striking differences in capacity to respond were disclosed among the several varieties. Statistical analysis shows that the widest difference in "test response totals," 87 to 125

Table II.—Tabulation of Acid "Test Intensity Totals." The Individual Entries Are "Test Intensity Totals" for the Three Plants Sampled from Each of the 96 Plots on the Date Indicated. The Totals in the Column Headed "Variety Grand Totals" Are the Sums of the Individual Variety Totals for the Three Test Occasions. The Indicated Fertilizers are "N"—Nitrogen, "P"—Phosphorus, "K"—Potassium

Variety	Fertilizer Treatment: None	N	P	K	NP	NK	PK	NPK	Variety Sub-totals	Variety Grand Totals
First Sampling—June 18, 1938										
Chinese	0 3	2 3	3 3	1 3	2 1	3 3	4 1	0 4	36	
Manchurian	6 6	3 3	6 1	0 3	3 3	1 2	1 0	5 7	50	
Italian	2 2	1 0	2 3	5 5	2 2	1 2	2 1	3 3	36	
Roumanian No. 1	3 3	2 0	6 2	3 1	0 1	2 0	0 4	1 2	30	
Roumanian No. 2	0 2	3 3	1 1	0 2	1 2	0 3	1 1	2 3	25	
Roumanian No. 3	3 0	4 2	3 2	2 5	4 3	0 1	3 0	0 0	32	
Fertilizer Sub-totals	30	26	33	30	24	18	18	30		
Age Total									209	
Second Sampling—June 30, 1938										
Chinese	4 3	3 1	4 3	4 3	2 5	0 2	4 5	6 3	52	
Manchurian	3 4	3 2	5 3	2 6	3 2	1 5	1 1	3 2	46	
Italian	3 0	3 1	2 4	2 0	4 2	3 2	2 3	3 3	37	
Roumanian No. 1	2 4	2 3	4 5	3 4	1 4	3 1	3 4	3 5	51	
Roumanian No. 2	2 3	4 3	3 2	1 2	4 2	3 3	3 3	1 4	43	
Roumanian No. 3	3 2	4 0	3 3	0 2	0 4	1 2	2 2	3 2	33	
Fertilizer Sub-totals	33	29	41	29	33	26	33	38		
Age Total									262	
Third Sampling—July 29, 1938										
Chinese	1 2	2 1	1 0	4 1	0 4	3 2	1 1	0 4	27	115
Manchurian	1 0	7 3	2 0	0 3	2 1	0 1	2 3	0 4	29	125
Italian	0 1	3 0	1 1	0 4	0 1	0 0	0 2	0 1	14	87
Roumanian No. 1	0 3	1 3	2 3	0 2	1 3	5 2	2 2	0 4	33	114
Roumanian No. 2	2 0	1 3	7 0	2 2	4 5	1 2	3 4	1 3	40	108
Roumanian No. 3	0 3	3 3	3 2	0 1	4 1	1 2	3 2	3 5	36	101
Fertilizer Sub-totals	13	30	22	19	26	19	25	25		
Age Total									179	
GRAND TOTALS	76	85	96	78	83	63	76	93		

Table III.—Computations Obtained by Analysis of Variance. The Designations of Contributors Are: "V"—Varieties, "D"—Dates Harvested, "N"—Nitrogen, "P"—Phosphorus, "K"—Potassium

Variance	D/F	Alkaline Beam Test: Mean Squares	F: Found	F: Required 99:1	F: Required 19:1	Acid Beam Test: Mean Squares	F: Found	F: Required 99:1	F: Required 19:1
Whole Plots									
Block	1	4.0138				3.5556			
Variety	5	582.6917	256.78	3.45	2.42	3.5972			
N	1	0.2222				0.0139			
P	1	5.0138				7.3473			
K	1	1.1250				3.1250			
NP	1	0.0140				0.3472			
NK	1	0.1250				0.1250			
PK	1	0.0556				0.3472			
NPK	1	0.2221				10.1250			
VN	5	2.6472				2.3639			
VP	5	0.7722				0.9805			
VK	5	1.8833				2.9083			
VNP	5	1.4389				1.6139			
VNK	5	1.3833				2.2083			
VPK	5	1.6472				1.2805			
VNPK	5	1.1472				1.1583			
Error (*a*)	47	2.2692				2.8959			

Table III (*Continued*)

Split Plots									
Dates	2	26.4479	13.69	4.85	3.10	18.3993	10 13	4.83	3.10
D Block	2	15.3368	7.94	4.85	3.10	0.8367			
DV	10	7.1771	3.71	2.72	2.04	4.8201	2.65	2.72	2.04
DN	2	0.7743				3.6909	2.03	2.72	2.03
DP	2	0.3577				1.9201			
DK	2	1.0729				0.5104			
DNP	2	1.6284				2.6701			
DNK	2	0.5104				4.1354			
DPK	2	1.3159				0.1701			
DNPK	2	0.0868				0.9479			
VDN	10	0.6613				2.6534			
VDP	10	3.3784				2.6159			
VDK	10	1.5937				0.8812			
VDNP	10	1.5909				3.1243			
VDNK	10	3.7062				4.1312	2.28	2.72	2.04
VDPK	10	3.1951				3.4159			
VDNPK	10	1.0243				0.7687			
Error (*b*)	94	1.9325				1.8155			
Total	287	12.4930				2.4494			

(Italian-Roumanian No. 2), is probably not due to chance variation, the odds being greater than 19 to 1 but less than 99 to 1.

Far greater were the differences found with respect to age. The test response total was greatest on the second occasion and, rather surprisingly, declined on the third to a value smaller than that of the first. The analysis presented in Table III shows that the variations were statistically significant.

Fertilizer treatment was found, as in the alkaline test, essentially without effect on the results.

Sex variations (Table IV) proved to be similar to those found for the alkaline test, that is, without significance when all varieties are considered as a whole. In the case, however, of the individual varieties, Roumanian No. 2 and Chinese, differences between responses of males and females were, respectively, 3.8 and 3.7 times the probable error. This difference is probably not due to chance variation, the odds being approximately 95 to 1. Since these rather striking differences are balanced by opposite differences in the other varieties the question of sex effect can scarcely be considered settled but it is plain that any real difference must be small.

Table IV.—Results Showing the Male and Female Plants Separated for Their Response to the Beam Tests

Variety	Number Plants	Test Intensity in Per Cent 0	1	2	3	4	5	Mean	P. E.	Difference P. E. Male	Female
Alkaline Test											
Males											
Roumanian No. 1	55		1.8	10.9	74.5	12.7		2.98	±0.05		
Roumanian No. 2	50			10.0	68.0	22.0		3.12	±0.05		
Roumanian No. 3	47		2.1	6.4	80.8	10.6		3.00	±0.05		
Italian	66		3.0	18.2	63.6	15.2		2.91	±0.06		
Manchurian	71	35.2	36.6	25.4	2.8			0.96	±0.07		
Chinese	61	34.4	52.5	9.8	1.6	1.6		0.84	±0.07		
Females											
Roumanian No. 1	89		3.4	6.7	64.0	24.7	1.1	3.13	±0.05	−0.15	±0.07
Roumanian No. 2	94			8.5	55.3	31.9	4.3	3.32	±0.05	−0.20	±0.07
Roumanian No. 3	97			8.2	67.0	22.7	2.1	3.19	±0.04	−0.19	±0.06
Italian	78		3.8	23.1	71.8	1.3		2.71	±0.04	0.20	±0.07
Manchurian	73	32.9	53.4	9.6	4.1			0.85	±0.06	0.11	±0.09
Chinese	83	45.8	41.0	9.6	3.6			0.71	±0.07	0.13	±0.10
Acid Test											
Males											
Roumanian No. 1	55	43.6	49.1	7.3				0.64	±0.06		
Roumanian No. 2	50	44.0	52.0	4.0				0.60	±0.05		
Roumanian No. 3	47	42.6	51.1	6.4				0.64	±0.06		
Italian	66	37.9	57.6	3.0	1.5			0.68	±0.05		
Manchurian	71	32.4	43.7	23.9				0.92	±0.06		
Chinese	61	23.0	59.0	18.0				0.95	±0.06		
Females											
Roumanian No. 1	89	30.3	50.6	19.1				0.89	±0.05	−0.25	±0.08
Roumanian No. 2	94	29.8	58.5	10.6	1.1			0.83	±0.04	−0.23	±0.06
Roumanian No. 3	97	36.1	54.6	9.3				0.74	±0.04	−0.10	±0.07
Italian	78	50.0	46.2	3.8				0.54	±0.04	0.14	±0.06
Manchurian	73	35.6	49.3	12.3	2.7			0.82	±0.06	0.10	±0.08
Chinese	83	38.6	54.2	7.2				0.69	±0.04	0.26	±0.07

CONCLUSIONS

The experiments herein described lead to the following conclusions:

1. Different agronomic varieties of Cannabis vary markedly in chemical makeup of resin as evidenced by wide differences in response to the alkaline Beam test.
2. Treatment of the soil with various fertilizers alone and in combination is without effect on alkaline Beam test response under the conditions studied.
3. Male and female plants respond essentially alike to the alkaline Beam test.
4. Intensity of response to the alkaline Beam test tends to increase with age of plants at least until the time of flowering.
5. Intensity of response to the acid Beam test was not definitely influenced by varying variety, fertilizer, sex or age of plants except that there was observed a statistically significant diminution in intensity after the plants had flowered.

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Polyploidy in Relation to Chemical Analysis

*By Wm. J. Bonisteel**

Polyploidy is present in nearly one-half of the flowering plants. Economic plants that are polyploid in nature include apples, wheat, oats, corn, tomatoes, tobacco (1) and many other plants that are of horticultural interest. Polyploids are organisms that have more than two sets of homologous chromosomes. Cytological and genetical studies show that interspecific hybridization, chromosome duplication and meiotic aberrations have played an important part in the development of new and improved forms of plant life. The function that polyploidy exerts in the chemical constituents in the realm of drug plants seems to have been largely overlooked up to the present time. By the use of chemical substances it is possible to induce polyploidy in certain plants (2). Sterile triploids may have their chromosomes doubled and thus become fertile plants. Polyploid plants are generally characterized by larger size, vigor, hardiness and larger cells.

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Polyploidy and Plant Constituents.—Plant breeding means the production and maintenance of new types through the direction and control of the natural processes of fertilization (3). Digitalis may be cited as a type that is carefully selected and bred for uniform constituents. That polyploidy induces chemical changes in the cellular constituents of plants has been shown by Crane and Zilva (1933) in their studies on the vitamin C content of tetraploid tomatoes which is approximately double that in the diploid plant from which they were derived. In the apple considerable variation is found in the vitamin C content between diploid and triploid varieties. Mangelsdorf and Fraps (4) have shown the effectiveness of genic changes in the production of the nutritional biochemical vitamin A when suitable pollinations are made in corn. Each gene for yellow induced approximately 2.5 units of vitamin A per Gm. of seed and they were able to increase the vitamin A content from 0.05 unit to as high as 5.00 units per Gm. Beasley (5) found that in tetraploid cottons doubling the chromosome number gave octoploids and the fiber length was increased. Such results show that a definite quantitative relationship exists between the genetic factors in a plant and the chemical substance produced.

Polyploidy and Toxicity in the Genus Aconitum.—In a previous paper (6) Sparks Aconite was investigated cytologically and this clone was found to have two somatic chromosome numbers. The majority of the root tips have the triploid ($3n = 24$) chromosome number but sectors were found in a few root tips which had the hexaploid ($6n = 48$) chromosome number. Thus Sparks Aconite is a member of the polyploid series of aconites. Preliminary tests indicate that this clone was toxic. It is to be noted that if a sector with 48 chromosomes reached a growing point a new type of plant would result. Since Sparks Aconite is a sterile clone and has never been known to produce seeds in nature or under adequately controlled hybridization pollinations such a 48 chromosomed plant would be fertile and produce viable seeds. Whether such a plant would be toxic or not is undetermined at the present time. Experiments now being conducted show that aconites can be induced to double their chromosomes by suitable treatment with colchicine. Treated plants show the characteristic stunted growth, thick heavy leaves and swellings indicative of chromosome doubling as a result of colchicine treatment.

A study was made of the chromosome numbers of several types of aconites and this was compared with the published chemical analysis and the assumed

toxicity. It is necessary to recall that it is not always possible for the cytologist and the chemist to be dealing with the same type of plant. Cases are known where *Aconitum Fischeri* was used for what was supposed to be *A. napellus*. Nevertheless the tabulated results in Table I show considerable uniformity as the results indicate. When diploid plants are compared, it is generally found that the plants are non-toxic. Published results are not available for all the plants tested and in those cases preliminary tests for toxicity were made without a series of adequate controls. But these results compared favorably with those plants which had seemingly well-authenticated chemical analysis.

lished records. In a series of hybridization experiments over several years it has not been possible to duplicate this plant. Over 30 different plants have been grown that were procured as true *A. napellus* species, but when critically studied showed extreme variation. Many failed to set seed and pollen abortion was relatively high indicating a high degree of unstability as far as seed production is concerned. Many of these plants are clones and only by the careful examination of the type specimen is it at all possible to determine the present status of the many so-called *A. napellus* types. The type specimens (in Europe) are now buried so that they will be safe for the future.

Table I.—Polyploidy and Toxicity in Certain Species or Clones of *Aconitum*

Species or Clones	Chromosome Number	Type	Chemical Structure	Toxicity
A. heterophyllum	16	Diploid	$C_{22}H_{35}O_8N$	Non-toxic
A. paniculata	16	Diploid	$C_{29}H_{33}O_7N$	Non-toxic
A. variegatum	16	Diploid	.....	Relatively non-toxic
A. noveboracense	16	Diploid	.....	Relatively non-toxic
A. napellus	24 (?)	Triploid	$C_{34}H_{47}O_{11}N$	Very toxic
Sparks Aconite	24 Clone	Triploid	.....	Considered toxic[a]
A. Stoerkianum	24 (?)	Triploid	..... (?)	Very toxic
A. napellus	32 (?)	Tetraploid	$C_{34}H_{47}O_{11}N$	Very toxic
A. chinense	32	Tetraploid	Aconitine (?)	Very toxic
.....	(40)	Pentaploid	Not observed in nature	
Sparks Aconite[b]	(48)	Hexaploid	Not observed as yet	
A. palmatum	48	Hexaploid	Palmatisine (?)	Non-toxic
.....	(56)	Septaploid	Not observed in nature	
A. Wilsoni	64	Octoploid	.....	Undetermined
A. Delavaji	64	Octoploid	.....	Undetermined

[a] Preliminary tests indicate toxicity.
[b] Hexaploid sectors in root tips if extended into growing points would produce a fertile plant from the sterile triploid clone.

The surprising fact is that the triploid and tetraploid aconites are very toxic. In this category is found aconitine. Some doubt exists as to the exact chemical composition of this alkaloid. Based upon the findings of cytology it may be noted that *A. napellus* seemingly has 24 chromosomes and also 32. Are these two different plants? This question can only be answered by an adequate cytological study. In the case of Sparks Aconite cytological studies show that the 24 chromosomes can be resolved into three sets. The parentage is not known. But from a critical analogy with other known plants it is assumed to be a hybrid arising from (1) two diploid plants in which one gamete was unreduced or (2) one of its parents could have been a tetraploid (double diploid) and the other a diploid. The problem is complicated since the parents of this clone are not known. On the assumption that part of its inheritance was diploid in origin is it to be assumed that the relatively non-toxic atisine type of aconites characterized by the diploids gave rise to a very toxic alkaloid? Some factorial change may result in the production of new molecules or transferring other radicals into new and more toxic positions. Does doubling of chromosomes confer toxicity on a relatively non-toxic plant? Based upon the data presented the evidence points in that direction.

A. Stoerkianum (given the status of a species) is evidently a hybrid between *A. variegatum* and *A. napellus*. The plant is toxic according to the published results on the cytology of the genus Cinchona

Only one hexaploid aconite has been examined chemically and it is considered to be relatively non-toxic. The alkaloid is palmatisine from *A. palmatum*, a plant growing in India. It would be mere speculation to guess at the relationship of this plant. It could have arisen as a result of chromosome duplication of a triploid. The plant is known to set viable seeds. Induced polyploidy in Sparks Aconite would produce a hexaploid. Such a hexaploid form is now being synthesized by suitable chemical treatment. Its toxic action cannot be predicted but it will be interesting to see if doubling a triploid will result in reduced or increased toxic action. Based upon the evidence from *A. palmatum* such a plant would be relatively non-toxic.

Plants with 40 and 56 chromosomes have not been found in the aconites growing in the wild. It is possible, however, to synthesize them if such were desirable. It has been possible to produce nearly a hundred types of aconites from the available breeding stock at hand. These plants possess all the characteristics that are present in the polyploid plants.

The octoploid aconites have not been investigated and there is no criterion to guide us except the cytological findings. Preliminary tests for toxicity were unsuccessful in this group, and material available was not sufficient for further study.

Cinchona Alkaloids and Hybrids.—Useful pub-

are very meager but it is to be assumed that considerable work has been done and that it will never be published. Whether the Americas will awake to the need of this important plant-breeding problem in sufficient time to prevent a shortage of this drug is indeed questionable. The chemist will undoubtedly be able to make synthetic quinine but the product has not yet reached the flow sheet stage in industry. From a study of the Columbian cinchonas, the writer found that many of the types were of such a nature that under suitable breeding programs plants could be produced that would yield high alkaloidal products. It is to be recalled that the parents of all high-breeding cinchona stocks were derived from the native cinchonas of the Andean highlands. The data in Table II show the hybrid yield of two cinchona crosses. The first hybrid, which is a cross between *Cinchona succirubra* and *C. officinalis*, actually gave a lower percentage of quinine than either of the parents. Cinchonidine is increased over the yield in either parent. Cinchonine shows but little difference and the total yield of all alkaloids seems but little changed. It is obvious that such a cross is undesirable but it does indicate that some genetical factors are responsible for this condition.

Table II.—Results of Alkaloidal Yield in Hybrid Cinchonas *vs.* Parents. Only Three Important Alkaloids Considered

Species or Hybrids	Quinine, %	Cinchonidine, %	Cinchonine, %	Undet., %	Total Alk., %
C. succirubra	1.91	1.14	2.11	0.85	6.04
C. officinalis	2.77	0.39	1.57	0.66	5.39
Hybrid of above parents	1.43	1.58	1.58	0.81	5.40
C. succirubra[a]	2.54	2.05	2.14	1.59	8.32
C. Ledgeriana[a]	3.81	0.00	0.04	1.15	5.00
Hybrid of above parents	6.77	0.73	..	..	11.83

[a] Cultivated species.

The cross between *C. succirubra* (7) and *C. Ledgeriana* gave a hybrid whose yield of quinine was nearly 100 per cent higher than either parent. These plants were cultivated and naturally did not have to compete with other plants for food. This is an excellent example of the marked heterosis that is often shown in hybrids. The low percentage of cinchonidine present in the hybrid shows that some factor in the chromosome complex shifted part of this molecule toward the quinine group. Since factors are grouped according to their most conspicuous effects it is proposed that the genes which exist in this hybrid should be called the genes intensifying quinine. The total yield of alkaloids in this hybrid is high but it is known that much higher yields have been produced.

Similar results have been obtained in the breeding experiments with the genus Aconitum. Many hundreds of crosses tried have been failures. Many of the failures have been due to abnormal meiotic behavior and as such do not fall within the scope of this paper. On the other hand some successful crosses have proved to be worthless plants. Still other plants have excelled the parents in the vigor of the stock, period of flowering and hardiness. Preliminary tests for toxicity indicate that in many of these hybrids it is possible to increase the toxicity of the aconites.

In the recent monograph published by the AMERICAN PHARMACEUTICAL ASSOCIATION on the genus Aconitum no mention was made of the chromosome number. Neither were any references made to the cytology of this group which clearly pointed out many irregularities which must be taken into consideration in this group with so many differing alkaloidal complexes. Many brilliantly executed papers have been published on the various phases of the aconite problem but one is never certain of the exact aconite that was used in the experiment.

Colchicine and Induced Polyploidy.—The alkaloid colchicine has the remarkable property of inducing polyploidy in certain plants. Seeds, buds and other parts of the plant are treated with water solutions with from 0.05 per cent of colchicine to as high as one per cent for varying periods of time. Solutions may be prepared in agar or in lanolin mixtures. The details of this procedure are adequately covered in the literature (2) and need not be repeated.

In the experimental work with the aconites the plant is extremely stunted by colchicine and shows only a few leaves while controls will often reach a height of 0.5 meter. The leaves of the treated plants are variously deformed and have a thick leathery appearance. Many of the plants die but this has been the fault of the experiment in using solutions that were too strong. It is necessary to vary the solution with each type of plant. The pollen is also an index of the induced polyploidy for the successfully treated plants have larger pollen grains. While certain aconites respond to the action of colchicine others do not respond to any normal concentration.

Colchicine and Double Diploids.—The simple explanation of the action of colchicine upon the cell may be inferred from treating a diploid aconite species with its normal complement of 16 chromosomes. In ordinary cell division there would be in the somatic tissues two cells each with 16 chromosomes. In the colchicine-treated plants the chromosomes are doubled since the mechanism for the separation of the chromosomes is arrested and the resulting cell has 32 chromosomes. This is a tetraploid plant or, in terms of the newer terminology, a double diploid.

In the cytological investigations with Sparks Aconite cells were found with 24 chromosomes in the root tip. In Fig. 1 the somatic chromosomes of this triploid are illustrated.

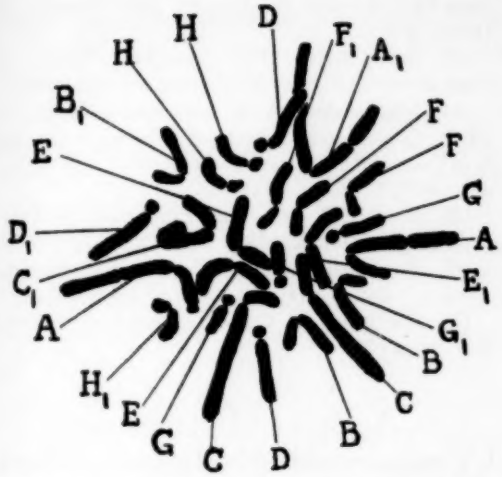

Fig. 1.—Somatic Chromosomes from the Root Tip of Sparks Aconite. Fixed in La Cour's 2BD and Stained in Newton's Iodine Gentian Violet ($3n = 24$).

In Fig. 2 the semi-diagrammatic sketch shows the relative size and shape of the three sets of chromosomes that are found in the root tips of Sparks Aconite. The detailed description of the size, shape and constrictions present in the three sets of chromosomes is omitted in this paper. However such information is necessary for any cytological investigation.

Fig. 2.—Semi-diagrammatic Drawings Showing the Relative Size, Shape and Constrictions Present in the Chromosomes from the Root Tips of Sparks Aconite. The Two *A* Sets Are Alike While the A_1 Set Differs in Length of Arms, Extended Knob at Tips and in Compactness.

In Fig. 3 the chromosomes found in the somatic cells with the hexaploid number of chromosomes is shown. It is to be noted that in the diploid three sets of chromosomes are designated *A*, *A* and A_1, etc. In the hexaploid these chromosomes are found doubled, that is, they are *AA*, *AA*, *AA*, *AA*, A_1, A_1, A_1, A_1, etc. This is exactly what happens when colchicine is effective in causing chromosome duplication.

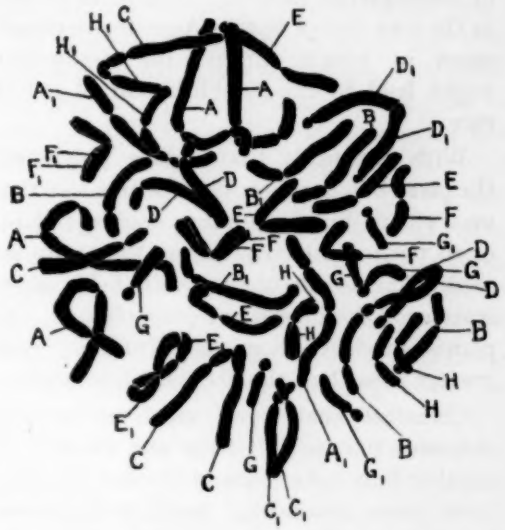

Fig. 3.—Somatic Chromosomes in a Single Cell from a Sector in the Root Tip of Sparks Aconite Which Have the Hexaploid ($6n = 48$) Number of Chromosomes. Cytological Technique as Indicated in Fig. 1. The Chromosome Duplication in This Clone Is Exactly What Takes Place When Colchicine Induces Polyploidy in Certain Plants.

In the plant world doubling of the chromosome number has occurred rather infrequently but one might have to wait a lifetime for this to occur. In Sparks Aconite this was found under experimental conditions as a normally occurring phenomenon. As has been indicated a growing point of this plant with a chromosome sector of 48 chromosomes could give rise to a new type of plant. Based upon other factors this plant would be fertile while the original triploid is sterile. Experiments to synthesize this hexaploid plant by means of colchicine treatment are now in progress.

SUMMARY AND DISCUSSION

In the genus Aconitum toxicity of the plants varies with the chromosome number. Diploids are usually non-toxic while the triploids and tetraploids represent the extremely toxic alkaloids of the genus. Hexaploids are considered non-toxic and no defi-

nite information is available about the octoploid forms. All the forms discussed are normally found growing wild with the exception of the triploids and these are often found wild as escapes from gardens.

Cytological evidence indicates that many of the triploids are plants in which diploids may have been the parents. It is to be assumed that many of these plants arose through chromosome duplication in cases of incompleted mitosis. Hexaploid sectors in the root tips of Sparks Aconite are clearly cases in which chromosome duplication might lead to the establishment of a new race.

With chemicals that induce polyploidy the plant breeder is provided with a tool that may enable him to produce double diploids or to treat sterile triploids so they may set seed. This is one of the fertile fields of research for the breeder of drug plants. The plants produce chemical substances with greater ease than the chemical laboratory.

Chemical analysis of cinchona hybrids indicates that a shift from one alkaloid to another may take place in the hybrids that have been produced. Such changes are known to occur in other plant products. In this, as yet unexplored, field some fundamental answer must be sought to explain the change that takes place in the chromosome mechanism which underlies these shifts in radical position or that exerts so profound an effect as shown in toxicity. It is suggested that the chromosome number be considered in the chemical analysis of these plants with a polyploid series of chromosome numbers. The status of the plant should be carefully considered. This is imperative with the problem of induced polyploidy through chemical treatment of plants. In the future all monographs published by the AMERICAN PHARMACEUTICAL ASSOCIATION should consider the cytological problems that are encountered in the preparation of monographs of drug plants.

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A Brief History of Commercial Medicinal Plant Culture in the United States*

By A. F. Sievers†

The desire to grow medicinal plants for the market comes to people of many classes, occupations and circumstances. Farmers, urban workers and business men at times become interested in such an undertaking, as evidenced by the hundreds of letters received annually by the U. S. Department of Agriculture requesting information on the subject. In most cases these inquiries are prompted by the belief that medicinal plant culture is a new and profitable enterprise. The high cost of medicines naturally suggests to many people that the plants furnishing the products that go into such medicines must be of high market value. Quite the contrary is the case. Botanical drugs, on the whole, are quite cheap and therein lies the principal reason why medicinal plant culture is not a well-established agricultural industry in this country. With few exceptions, our requirements of the crude drugs come from one of two sources—they are imported from abroad or they are collected from domestic wild plants. Many come from the tropics where they are grown or collected with very cheap labor.

Interest in the subject is usually greatest in periods when persons are more or less dissatisfied with their lot or forced to seek new means of a livelihood. During depres-

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sion years and when returns from staple crops are especially unsatisfactory there is a tendency to turn to such endeavor, usually with a serious misconception regarding the difficulties involved and the profits that may be expected. The period of greatest interest in the past was during the World War when the prices of crude drugs of foreign origin rose to levels previously unknown. A somewhat similar situation is again at hand and war conditions in central Europe may once more create a period of scarcity and high prices of many important botanicals coming from that region. Already the quotations on some items are substantially higher than normal and interest in the domestic cultivation of these is increasing daily. Not only are people considering their culture but manufacturers of preparations in which drugs and related plant products from foreign sources are used are becoming concerned about their supplies of these raw materials when present stocks are exhausted.

Many species now imported are adapted to some sections of this country and fortunately past experiments and studies by the Department and other agencies have provided much useful information on the soil and climatic requirements of such plants, methods of their culture, labor requirements, yields, markets, etc. One of the chief difficulties is the procurement of seed and other planting stock sufficient for even a relatively small acreage. It is believed that attempts to grow such plants should be limited to the few species that are most indispensable and have the best established market. Digitalis, belladonna and henbane, all leaf drugs, are well adapted to culture, have long been firmly established in medical practice and were most successfully grown in the past. To these might be added some of those from which are obtained the aromatic seeds such as anise, fennel, coriander, celery and others, and the condiments sage, marjoram, tarragon, etc. Among the species yielding volatile oils, geranium and lavender have possibilities but the latter requires several years to come into production.

It is advisable that those who contemplate the growing of such plants inform themselves regarding the experiences of others who engaged in this enterprise in the past. Some of the difficulties encountered will doubtless be met with again but some mistakes made can perhaps thus be avoided. With this in mind the following brief history of the most important attempts at commercial medicinal plant culture in the United States is presented.

The possibilities of medicinal plant culture in the United States have been profoundly affected by changes and trends in the practice of medicine and in our economic and social life. The basis of the so-called herb-growing industry of colonial days and of much of the nineteenth century was the use of savory or flavoring herbs in cooking and of other herbs as components of medicinal teas and related preparations for which the pressed herbs of the times were largely used. The first-mentioned use still exists and the growing of savory herbs in the garden and in a limited way for sale in city markets continues and is in fact increasing. Commercial sage culture was probably the best example of this branch of the industry. The use of medicinal teas has undoubtedly declined to a mere fraction of what it was at one time. The business in pressed herbs was still considerable at the turn of the present century but soon thereafter many that formerly found a ready market could not be sold. The market became restricted largely to manufacturers of galenicals and proprietaries of the day and the sale of pressed herbs over the drug store counters became yearly less important.

The most successful of the early endeavors to grow and market medicinal plants were community enterprises, for which our present social order is generally not so well adapted. Reference is to the various Shaker Societies that flourished for a while in the nineteenth century in which the growing and collecting of a variety of flavoring and medicinal herbs were successfully accomplished. Such societies existed in numerous communities in New England, New York and elsewhere in the East and Middle West. Those interested are referred to the literature on the subject such as "Gleanings from Old Shaker Journals," published in 1916 by C. E. Sears, which contains many items of interest. Some

of these Shaker Societies marketed herbs valued as high as $8000 in a season and collectively the annual volume of business was quite large for those days. They not only cultivated and collected the crude drugs but converted them into various products. A catalog issued by the United Society of Shakers at New Lebanon, New York, states that this society was first established in 1800 and lists "Medicinal plants, barks, herbs, seeds, flowers and select powders: therapeutic extracts, ointments, inspissated juices, essential oils, double distilled and fragrant waters."

With the passing of these early activities there followed a period during which the cultivation of botanical herbs appears to have declined almost to the vanishing point, especially so far as concerns the great majority of the species previously cultivated. Regarding these there appears to be little information on what occurred up to the beginning of the World War although it is known that some experimental work with these was going on. Commercial growing of a few plants such as ginseng, goldenseal, wormwood, peppermint and American wormseed has continued without interruption for many years.

No reliable statistics are available on the amount of ginseng and goldenseal root produced from cultivated plants. Since numerous persons grow both and their cultural requirements are somewhat alike, they are usually considered companion crops although ginseng root is grown only for export whereas goldenseal root and herb are used in this country to the extent of probably several hundred thousand pounds annually. There are probably not more than several hundred acres of these plants. Small plots, many of them less than an acre, are scattered throughout the northeastern, northcentral and Pacific Coast states. The growers of these plants have experienced many difficulties and few of those who undertake their culture bring their plantings successfully through the four- to seven-year period required to mature their roots. Severe declines in market value have discouraged many others. Goldenseal root declined from four dollars to fifty cents a pound within a few years and the normal exporting of ginseng to China, where its principal market lies, is interfered with during periods of disturbed conditions there.

The culture of peppermint as a source of the herb has never been extensive, but as a source of oil this plant has been grown in the United States for more than a century. The crop was introduced in central New York but as early as 1835 the plant was found to be well adapted to the black muck soils of northern Indiana and southwestern Michigan and for many years about 90 per cent of the American peppermint oil has been produced in that area. About 25 or 30 years ago mint culture was started on similar soil types in western Oregon and Washington, which region now furnishes the balance of the domestic production. No peppermint oil has been produced in New York State for some time. Spearmint is grown as a companion crop but in much smaller amounts. In 1938 there were 29,070 acres of mint under cultivation which yielded over 890,000 pounds of oil. Nine-tenths of this was produced in the Midwest. The average yield per acre was about 31 pounds and the average price received about $2.00 per pound. Growers claim this is below the cost of production. About 12 years ago several short crops resulted in very high prices, oil being sold in some instances for as high as $25 a pound. The inevitable overproduction followed immediately with the usual consequences.

Japanese peppermint, the oil of which is the only commercial source of natural menthol, and which is cultivated extensively in Japan, was grown to the extent of several hundred acres in central California for several seasons within the past fifteen years. When grown under irrigation this crop was cut two or three times in a season and the season's yield of oil was about 60 pounds although as much as 200 pounds to the acre were obtained in some cases. This oil contained from 75 to 80 per cent of uncombined menthol. The crop was grown largely under contract, which permitted a reasonable profit. However, these contracts could not be renewed on the same price basis after menthol prices declined and the crop was soon abandoned.

Perhaps the most interesting essential oil crop in the country is the American wormseed which has been cultivated for the production of chenopodium oil, a well-known anthelmintic, for more than 100 years in a small area in central Maryland. It is a curious fact that the crop never became established elsewhere but this may be due to the erroneous belief that once prevailed that oil produced in any other region is lacking in quality. In later years experiments in South Dakota and Illinois have indicated that in these places good oil can also be produced. According to the 1930 census there were at that time 139 growers with 368 acres which produced 15,000 pounds of oil. Prior to that its production was frequently much greater, especially in 1924 when 600 acres were grown. The industry, once fairly prosperous, has in recent years suffered continuously from overproduction, largely because acreage has not been adjusted with regard to the declining consumption of the oil due to increasing use of other anthelmintics.

Another essential oil crop that requires mention here is wormwood. Many years ago wormwood was cultivated in Wisconsin where the oil was distilled with crude equipment. Since then its culture has

become more confined to the midwest where mint growers cultivate small acreages from year to year, in accordance with market conditions. The only important outlet for the oil is as a component of liniments, for which the amount required is relatively small. Wormwood has also been cultivated for the herb which at one time was in good demand. It is one of the crops given numerous trials during the World War period.

Other special plants in this general group have been grown for limited periods but various conditions and circumstances caused their cultivation to be discontinued. Space does not permit more than brief mention of these sporadic and widely separated attempts to grow such plants.

Sage was grown quite successfully at times, especially in Michigan and Wisconsin, from about the turn of the century to the War period. It was grown for the spice and meat-packing trade, however, rather than for medicinal purposes. About 30 years ago, there was considerable interest for a few years in drug plant culture in northern Texas where one individual, according to available records, cultivated a variety of plants with some success. Larger yields were claimed to be possible on account of the longer growing season but the long distance from the principal markets was a disadvantage. With the death of the most interested person the enterprise was abandoned.

In Wisconsin a unique project was undertaken in 1912 when the state legislature established the Wisconsin Pharmaceutical Experiment Station at Madison, Wisconsin. Researches on various phases of medicinal plant culture were undertaken but these plants were grown in sufficient quantity to constitute at least a semi-commercial undertaking. During the period of shortage of important drugs, quantities of digitalis of excellent quality were furnished to hospitals and institutions. Surplus materials were sold on the open market. The project did not survive.

In 1913 an interesting attempt at commercial drug plant culture was made at Omro, Wisconsin. Close coöperation between the grower and the Bureau of Plant Industry made it possible to secure detailed records. The experiences of the company are typical of that of others during that period. The plants grown were burdock, wormwood, spearmint, sage and belladonna. Five-eighths of an acre of burdock produced 480 pounds of roots and 100 pounds of leaves, the returns from which just covered the costs. From 8 acres of wormwood about $300 net was received. The crop was cut with a binder, the tops stripped by hand and sold as herb and the stems distilled. The herb furnished two-thirds of the gross returns. In another year 7 acres produced 3400 pounds of herb but only part of this could be sold. Spearmint was largely destroyed by rust and weeds. Sage appeared to be a promising crop, judged from the small plot grown, but the price at the time was 60 cents a pound. By far the most profitable crop was belladonna, half an acre of which brought a net income of over $400. Most of the crop was sold for $1.30 a pound. A change in the personal affairs of the owner caused this venture to be discontinued after 1915.

The rapid decline in the imports of such important botanicals as digitalis, henbane and belladonna with the beginning of the World War in 1914 led to the most active period in medicinal plant culture in our history. Not only were individuals attracted to this enterprise by the high prices of these drugs but manufacturers of medicines resorted to their cultivation to assure themselves of the necessary supplies. Some of the manufacturers had already experimented for years with such plants and, consequently, had a valuable background of experience. The literature of the period and unpublished reports show that such companies grew digitalis, cannabis,[1] belladonna and henbane in Minnesota, Michigan, Indiana, New Jersey and Pennsylvania. In most cases several acres of these plants were under cultivation. With the return of normal prices after the War these activities were largely discontinued but the growing of digitalis is being continued by some of these firms. Apparently this drug can be grown to better advantage than the others, perhaps because a drug of more uniform potency is thus assured. Cannabis appears to have been a rather satisfactory crop and several of these firms report having grown this until recent years.

Near Richmond, Virginia, a drug farm was started in 1916 after several years of experimenting. There were six acres of digitalis and smaller acreages of cannabis, belladonna and stramonium, also small plantings of catnip, wormwood, calamus and orris root. Only digitalis, which was yielding about 1000 pounds per acre, was continued after 1916 and is still being grown there.

Among the incorporated groups who were attracted to medicinal plant culture in that same period there were two that were not manufacturers of pharmaceuticals and who, therefore, did not provide their own market, yet they were the largest producers. In Virginia, near Washington, D. C., a company was organized for that specific purpose and continued in business from 1915 to 1920. During the period of its greatest activities this company had under cultivation 15 acres of belladonna, 10 acres of digitalis, 10 acres of cannabis and 5 acres of sage. There were also 1½ acres of woodland planted to ginseng and goldenseal but nothing was realized from these. During the period that imports were restricted the company realized good prices for belladonna, digitalis and sage but those interested in the project declared that overhead expenses and general operating costs in that region were too high to permit sustained profitable cultivation of these crops even at wartime prices except on a large scale.

The other of the two companies is a large grower of peppermint in Michigan. On its large farm in the southwestern part of the state this company had under cultivation in 1918, 44 acres of henbane, 9 acres of belladonna and 10 acres of burdock. Hen-

[1] See footnote 2 on page 412.

bane was their most successful crop. The yield was about 700 pounds of dry herb per acre and a total of about 40 tons of this drug was sold from the farm in three years. Officials of the company state that none of these crops could be grown at a profit when their importation was renewed after the war, hence all were discontinued after 1920. Separation of the leaves from the stalks in the case of henbane and belladonna, and the drying of these in structures suitable for the purpose are mentioned as the most important items of expense.

The cultivation of two species of Datura was undertaken in Minnesota at about this time by a company for use in a special product. Three or four acres were grown and the quality of the crop was very good. While production costs were moderate they, nevertheless, greatly exceeded the market value of the drug before the war and the venture was naturally discontinued with the return of normal prices. This company also experimented with these species in California and finally planted about 30 acres there but lost the entire crop through the ravages of beetles.

During this period of high prices belladonna seems to have aroused the interest of more people than any of the other plants mentioned. In addition to those cases already referred to, there were many individuals in various parts of the country who grew belladonna exclusively and with some success. The largest acreage of belladonna in any one region was that in California. In that state interest in the crop was largely due to Dr. Albert Schneider who had a lifetime interest in medicinal plant culture. The first planting of the crop in northern California was made by him. Space permits only a brief statement concerning the acreage, production, etc. In 1918, almost 100 acres were reported, which was more than twice the acreage reported from any other state. No complete records are available but some idea of the acreage and number of growers in the several states in 1918 may be obtained from the following tabulation based on information secured through questionnaires sent by the Bureau of Plant Industry in the late fall of 1918 to all growers whose names were on file.

Estimated Belladonna Acreage and Number of Growers in the United States in 1918

State	Number of Growers	Number of Acres
Michigan	13	45.5
Indiana	7	32.7
New Jersey	4	34.7
Pennsylvania	6	41.7
California	24	95.6
Maryland, Virginia, Illinois Wisconsin, Oregon, Iowa	6	23.3
Total	60	273.5

These growers reported a total harvest of 83 tons of herb, somewhat less than half of which was separated into leaves and stems at harvest time. Eleven tons of roots were also harvested. Since some growers undoubtedly failed to receive questionnaires or failed to make replies, the actual production for the year must have exceeded this amount.

During the period 1913 to 1919, Cannabis[2] was grown for the drug trade in the vicinity of Florence, South Carolina. According to available records, there was an increase of from 8 acres in 1913 to 30 acres in 1916, 1917 and 1918, and a decline to 6 acres in 1919. Thereafter the crop was completely abandoned. These acreages were distributed among several growers, most of whom grew only an acre or two. The crop was prepared for market during the winter by stripping the leaves and tops from the stems by hand, a tedious and expensive procedure Many difficulties were experienced in marketing the crop. Dealers objected to the product for various reasons at times and the limited and declining market clearly indicated the doubtful future of the crop. There has been no attempt to revive the industry since that time. Federal legislation enacted in 1937 prevents the growing of this drug crop in the United States.

Occasionally attempts were made in the past 20 years to grow Levant wormseed as a source of santonin in various sections of the country. The company operating in Virginia near Washington, D. C., already referred to, got the plant started well on rather poor soil after growing the seedlings in the greenhouse. However, the summer rains caused the plants to die from crown rot. The unripe tops contained a high percentage of santonin. An eastern manufacturer of pharmaceuticals had considerable success with a small-scale planting in the San Joaquin Valley in California, but the limited market for santonin discouraged any commercial planting. The Bureau of Plant Industry tried the plant in a number of localities and found it best adapted to irrigated regions in the West with little rain and high temperatures in summer. As a result of these trials a small company undertook the production of santonin in 1927 in northeastern Oregon, and this has been continued without interruption. Many difficulties were encountered in processing the crop, but a satisfactory extraction plant was finally developed. The growing of the crop under irrigation has given little trouble and the yield of santonin has been satisfactory. The very limited domestic consumption of santonin and its well-organized production in several European countries are factors that limit such an industry to a very small scale.

Although the amount of castor oil used for various technical purposes is far greater than the quantity used medicinally, the castor-bean plant, source

[2] The Marihuana Tax Act of 1937 imposes an occupational tax upon all persons who import, manufacture, sell, deal in or dispense marihuana (*Cannabis sativa*). Under this enactment marihuana may be transferred only pursuant to an order on a form issued in blank for that purpose by the Commissioner of Internal Revenue and upon payment of transfer tax at the rate of $1.00 or $100 per ounce, depending upon whether the transferee is registered or unregistered.

Each state has enacted legislation controlling the sale and distribution of cannabis and, in many cases, the production or cultivation of the cannabis plant.

of castor oil, which is used for both medicinal and technical purposes, was a cultivated crop of some importance in the Middle West during the period following the Civil War until about 20 years ago. Kansas and Oklahoma were the principal producing states and during the early period the beans were crushed in mills located in the Mississippi Valley. In Oklahoma the maximum acreage and production were 2549 acres and 22,481 bushels, respectively, during the early years of this century. According to annual reports of the Kansas State Board of Agriculture that state produced 766,000 bushels from 68,170 acres in 1879. In 1916, the last year included in these reports, only 360 bushels were produced. In 1918 an organized campaign for castor-bean culture was undertaken by the War Department, but the purpose was to supply castor oil for lubricating purposes and, hence, it need not be discussed here. There is at present no domestic commercial production of castor beans.

Two other crops should be mentioned briefly although here again the medicinal use of their products is altogether secondary. Mustard seed is produced in California and Montana in greatly varying amounts. In 1938 the production in these states was 2,600,000 and 15,000,000 pounds, respectively. The other crop is the pungent red pepper of which several varieties are grown in the South and Southwest. Here, as in the case of mustard, its use as a condiment provides the market outlet. From 5000 to 7000 acres, yielding about a million pounds, are grown in southern California, Arizona, New Mexico, Louisiana, Mississippi and South Carolina. These two crops are of some importance in the spice industry but are given only mere mention here because they are not usually considered in connection with medicinal plant crops.

Correlation of the Evaluation of Disinfectants by the Agar Cup-Plate Method and Clinical Experience*

By Rhett G. Harris† and William A. Prout‡

Recently the authors were asked to evaluate a series of germicides and determine their efficiency, by *in vitro* methods, for use in the obstetric wards of the Roper Hospital, Charleston, S. C. Usually the criteria for such recommendations are reached by performing tests based upon dilution of the substances under investigation and then determining the time required for the serially diluted material to destroy bacterial growth. Examples of such methods are the Rideal-Walker test, the well-known F. D. A. phenol coefficient and the tissue culture and manometric procedures. If a germicide must diffuse through mucous material to reach the site where its action is desired, as in the birth canal before and after parturition, it is felt by the present authors that methods based upon diffusion would be better than those based upon dilution and simulate more, *in vitro*, the actual conditions under which germicides are used in obstetrics. One such test, the agar cup-plate method which was devised by Himebaugh and published by Ruehle and Brewer in 1931, has been studied by Rose and Miller (1939) in detail, and has been found to yield consistently good results, providing standard procedures are followed. This test was therefore chosen to be used in these investigations.

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EXPERIMENTAL

Method of Procedure.—Sterile Petri dishes were poured with 20.0 ml. of melted, cooled nutrient agar (p_H 7.2-7.4) containing in suspension 0.10 ml. of a 24-hour nutrient broth culture of *Staphylococcus aureus*. The plates were allowed to harden after which time test "cups" were cut from the agar with a sterile cork borer having a diameter of 15.0 mm. To this "cup" was added 0.20 ml. of the solution under investigation; the plate was covered with a porous, unglazed porcelain cover, incubated at 37.5° C. for 24 hours at which time measurements of the zones of inhibition were made. The zones produced by the various germicides requested in this investigation are shown in Table I.

Table I.—Widths of Inhibition Zones Produced by Germicides

	Germicide	Zone of Inhibition, Mm.
1.	Merthiolate (Tr. 1:1000)	18.0
2.	Iodine (3.5% solution)	16.0
3.	Iodine (3.5% tincture)	12.0
4.	Mercresin	12.0
5.	Metaphen (Tr. 1:200)	9.0
6.	Mercurochrome (surgical)	9.0
7.	Mercurochrome (4.0% tincture)	8.0
8.	Mercurochrome (4.0% solution)	6.0
9.	Hexylresorcinol (ST 37)	5.0
10.	Amphyl (undiluted)	4.0
11.	Amphyl (2.0% solution)	3.5
12.	Amphyl (5.0% solution)	3.5

The question arising after the completion of such a series of tests is, obviously, of what significance are

the figures which represent the width of the zone surrounding the test cup? The order of the respective zones may be arranged, as above, to give the most and the least reactive germicides and also determine the relative positions of the intermediate substances. However, what relationship do they bear to one another? In other words, what relationship exists between zones, diffusibility and potency? If we look upon the agar cup-plate method in terms of the amount of material that is rendered sterile per unit volume of germicide, we will be approaching a figure that will give us more nearly the information that we desire. Such a figure, the coefficient of diffusion, may be determined by dividing the volume of material rendered sterile by the volume of germicide added. The latter figure will remain constant

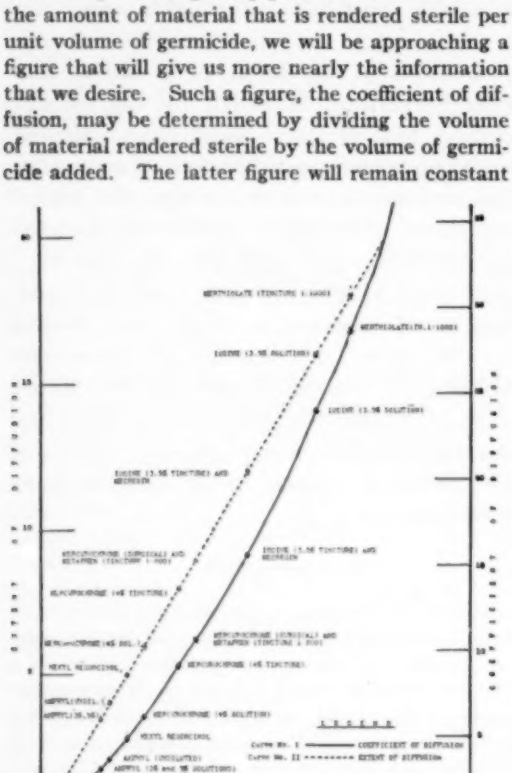

Fig. 1.—Curves Showing Relationship of Coefficient of Diffusion of Germicides to Extent of Diffusion.

in the agar cup-plate method at 200 cu. mm.; the former figure is calculated from the general formula

$$V = \pi h(r_1 + r_2)(r_1 - r_2) \qquad (1)$$

where V = volume of a cylindrical shell
h = altitude of the cylindrical shell
r_1 = radius of the outside ring
r_2 = radius of the inside ring

and is found to be, under the standard conditions of the test to become

$$V = 9.739.x(15 + x) \qquad (2)$$

where x = the width of the zone.
Substituting variables of x in equation (2) and plotting the corresponding calculated values of V against them, we obtain the curve (see Fig. 1) having the slope

$$dy/dx = 146.085 + 9.739.x \qquad (3)$$

which may be proved by differentiating equation (2).

If we calculate, for the substances tested, their respective coefficients of diffusion (see Table II) and place them on the curve plotted above (see Curve II, Fig. 1) we may see the relative position of one to the other. On the other hand, if we merely plot the extent of diffusion, in terms of the zone width, and place the data of Table I upon such a curve (see Curve I, Fig. 1) we note that the relative position of the germicides is much altered, especially in the cases of germicides having a narrow zone. This obviously leads to the overestimation of these substances. The extent of this error is found to decrease with increases in the width of the zone; thus this consideration decreases in importance in testing the more powerful germicides.

Table II.—Coefficients of Diffusion of Germicides

	Germicide	Coefficient of Diffusion
1.	Merthiolate (Tr. 1:1000)	28.92
2.	Iodine (Aq. sol. 3.5%)	24.15
3.	Iodine (Tr. 3.5%)	15.78
4.	Mercresin	15.78
5.	Metaphen (Tr. 1:200)	10.52
6.	Mercurochrome (surgical)	10.52
7.	Mercurochrome (Tr. 4.0%)	8.96
8.	Mercurochrome (Aq. sol. 4.0%)	6.13
9.	Hexyl resorcinol (ST 37)	4.87
10.	Amphyl (undiluted)	3.78
11.	Amphyl (2.0% solution)	3.15
12.	Amphyl (5.0% solution)	3.15

The results shown in Table II are of much interest in that there has been found a direct correlation between the efficiency, as calculated above, and their usefulness in the obstetric wards. No data will be presented in this preliminary report to substantiate this point inasmuch as a detailed study of each substance is now being undertaken in the hospital. The statement is based upon personal communications with Dr. Lester Wilson of the staff of the hospital in which the germicides have been in use, whose years of experience back up these results.

The figures are also of importance in that they show no correlation between the coefficient of diffusion and the Hg content[1] of the mercurials studied. This is congruent with the finding of Miller and Rose (1939) and is to be explained upon the characteristics of the individual germicides.

Most procedures based upon dilution place iodine low in the series, as Bronfenbrenner, *et al.* (1939), whereas in obstetrical practices, providing adequate nursing facilities are available, it is one of the best germicides we know. We find that iodine is more correctly evaluated when tested by this method, that is, placed high in the series superseded only by Merthiolate.

[1] Merthiolate 48% Hg, Metaphen 57% Hg, Mercurochrome 26% and Mercresin 58% Hg.

There is apparently no general relationship that exists between tinctures and solutions of germicides and their efficiency following diffusion, as shown by the conflicting results obtained with such preparations of iodine and mercurochrome. Topley and Wilson, generalizing, state: "Germicides dissolved in alcohol . . . are deprived of the greater part of their power." In this study this seems to hold true only in the case of iodine. Solutions of germicides containing soaps, such as Amphyl, apparently do not diffuse readily through collodial material. This is shown by their low coefficient of diffusion. Two factors probably are important in any consideration of such solutions; first, soaps alter the germicidal powers (Tilley and Schaffer, 1925, and Tilley, 1939) of germicides and, second, as noted in these experiments, the soap brought about changes in the colloidal gel itself thus preventing diffusion through it. Such solutions cannot be accurately judged by the agar cup-plate method, but the fact remains that Amphyl and its solutions have found little value in obstetrics where diffusion through mucous-like materials is desired.

A detailed study involving the various germicides mentioned in this treatise, as well as many others, is now being carried out in respect to diffusibility and clinical usefulness.

CONCLUSIONS

1. The coefficient of diffusion has been explained and its significance discussed.
2. Germicides having a high coefficient of diffusion have been found to be of value in obstetrics; those having a low coefficient were found to be of little value.
3. The agar cup-plate method is recommended especially for the evaluation of germicides where their use will involve diffusion through colloidal material.

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Problems Encountered in the Manufacture of Compressed Tablets*

By L. W. Busse and A. H. Uhl

The chief problems encountered in the manufacture of compressed tablets are binding, picking, capping, sticking and splitting. These problems are particularly annoying because they do not occur until the last step in the process of manufacture; namely, that of compression.

Capping and splitting are alike in the respect that they usually occur together and in many cases may be attributed to the same cause.

Capping is the term applied when the upper surface of the tablet splits off.

Splitting is the term applied when the tablet does not hold together after compression.

In discussing the remedies for capping and splitting, one must of course first know the reason for their occurrence. The reason given by many authors is that of excess powder in the granulation. This excessive amount of fine powder requires a great deal of pressure to form the tablet. It has been said, that this extreme pressure makes the tablet very hard in the center causing the upper surface of the tablet to split off. This fine powder also causes air to be trapped in the powder upon compression and after release of the pressure causes the upper surface of the tablet to split off.

Capping and splitting may also result from a worn upper punch, too much pressure or too damp or too soft a granulation.

It has been our experience, that in most cases capping and splitting were caused by

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having too soft a granulation. When this occurred all attempts to remedy these troubles, aside from regranulation, were unsuccessful. Reduction of pressure would yield too soft a tablet. Sifting out the fine powder using a number 30 screen was also unsuccessful. A worn condition of the upper punch was ruled out because in the majority of cases the set happened to be new. Regranulation, however, which resulted in a hardening of the granules most always succeeded in eliminating these troubles.

One of the most essential qualities of a granulation as far as compression is concerned is hardness. If you have a hard granulation, satisfactory tablets will most always result upon compression. This seems to be in accord with a statement made by Bentley, that the formation of the tablet on compression is assisted owing to the interlocking of the granules. The amount of fine powder allowed in the granulation also will depend upon the hardness of the granules. A hard granulation permits a higher percentage of fine powder than a soft granulation.

Tests were run on granulations containing various percentages of fine powder in order to determine at what amounts capping would be most likely to occur. These tests could not be carried out with any degree of accuracy, but in most cases thirty per cent of fine powder seemed to be the most satisfactory amount. However, granulations containing fifty and sixty per cent of fine powder were also satisfactory in some cases indicating the amount of fine powder is not always the cause of this trouble.

It was found that capping would also occur if not enough fine powder was present in the granulation. This would occur especially in the compression of crystalline substances such as methenamine, sodium bromide etc. Crystals or granules of a No. 16 mesh were purchased but it was found that these had to be forced through a No. 20 mesh screen to provide some fine powder with the crystals before they would compress satisfactorily.

In attempts to remedy these troubles once they occurred, the steps followed were (1) to reduce the pressure; (2) sift out the fine powder and regranulate. It was found also that the addition of $^1/_2$ to 1 per cent of stearic acid sometimes remedied these troubles, especially when they occurred with granulations prepared with powdered sugar.

Another method recommended by Moseley is to spray a 1 per cent solution of glycerin in 60 per cent alcohol on the granulation and allow to dry, then compress.

Binding, picking and sticking may also be grouped together for purposes of discussion because they most always occur at the same time and in many cases may be attributed to the same cause.

Binding is the term applied when the granulation sticks to the dye and causes a friction between the lower punch and the dye.

Picking is the term applied when the granules adhere to the face of the upper punch forming a concavity in the upper surface of the tablet.

Sticking is the term applied when the granules adhere to the face of the upper punch causing the surface of the tablet to be dull and pitted.

Binding, picking and sticking are usually the result of improper lubrication or using a granulation which is not quite dry.

Binding was found to occur quite frequently with substances such as sodium salicylate and calcium lactate. These substances were found to be very difficult to lubricate properly. The common lubricants such as talc, mineral oil, stearic acid, etc., were not satisfactory in amounts usually recommended. Soaps, however, were found to be very good as lubricants to prevent binding with sodium salicylate. Of the soap powders used, magnesium stearate in the impalpable powder form was found to be the most satisfactory. Three to four per cent of the total weight of the formula was required.

Calcium lactate was found to be lubricated equally well with talc or magnesium stearate. These were necessary in amounts of four per cent.

If the binding was the result of a granulation not thoroughly dry, it was found practically impossible to eliminate. Further drying was found to be the only satisfactory remedy. Mineral oil would remedy it for a

few minutes, but picking and sticking would also occur after operation of the machine for a few minutes so that the granulation would have to be dried.

Picking and sticking were found to occur most always with a granulation which was not quite dry. The first precaution, however, is to be sure that the upper punch is clean and highly polished. The use of chromium plated punches is now recommended as a precaution against the occurrence of these conditions.

It was observed that if these troubles occurred to any degree, they were almost impossible to eliminate. Talc served as a remedy in some cases, but a high percentage was usually required which was objectionable. Lubricants such as stearic acid, mineral oil, magnesium and sodium stearate are also objectionable when added in amounts necessary to overcome these difficulties. Stearic acid in amounts above four per cent retards disintegration. Mineral oil was found to decrease the binding properties when used in amounts above three per cent and as a result produces a soft tablet. The stearates if used in too large a quantity tend to produce sticking and picking in themselves.

In making this study it was observed that these troubles most always occurred with compounds such as aspirin, sodium salicylate or compounds which behaved similarly to those upon the addition of water. When water was used as the granulating liquid, the substances became pasty and sticky, making them difficult to granulate and also difficult to dry. When powdered sugar was used as the binder, which permits the use of high concentrations of alcohol in water as the moistening agent, these substances were very easy to granulate as well as to dry. As a result picking, sticking and binding with these substances was entirely eliminated.

One of the important observations made, during the last year, was the fact that these problems can be prevented much easier than they can be remedied. A thorough study of the proper moistening, binding and lubricating agents used to granulate the substance will in the majority of cases eliminate these difficulties encountered in the manufacture of compressed tablets.

Each substance must be studied as an individual compound and treated as such. Compounds when granulated with various binders and moistening agents exhibit different physical properties. This was observed particularly with sodium salicylate. All attempts to granulate this substance using aqueous granulating liquids were unsuccessful. However, the use of powdered sucrose and seventy per cent alcohol proved very satisfactory. When magnesium stearate was found to be a good lubricant all the problems encountered with this compound were eliminated.

With the compound aspirin formula, consisting of aspirin, phenacetin and citrated caffeine, all of the difficulties were encountered until it was found that the use of water as a moistening agent with aspirin was undesirable for the following reasons. One because of the hydrolysis which might occur and two, because of the difficulty in drying it thoroughly at a low temperature. These problems were greatly eliminated by using a crystalline aspirin and mixing these with the granules formed from the mixture of phenacetin, citrated caffeine and powdered sugar. In this manner the aspirin does not come into contact with water, and is added to the granulation along with the starch and the lubricant. This method is used for all formulas of two or more ingredients, which contain aspirin as one of the active ingredients.

Our experiences are presented with the hope that they will stimulate others engaged in the manufacture of tablets to present their problems and solutions so that we can all benefit from each other's experiences in this field. This will eliminate, to some extent at least, that problem which all teachers of tablet manufacturing are confronted with, namely, "practical experience" which is so necessary in the successful manufacture of compressed tablets.

"The more extensive a man's knowledge of what has been done, the greater will be his power of knowing what to do"—DISRAELI

The Progress of Pharmacy in Great Britain

*By Leslie G. Matthews**

"To be accounted a gentleman one must have ancestors."

The longer the line of ancestors the more honorable their descendant. The pharmacist of to-day in Great Britain in tracing his descent from the medieval apothecaries has some regard for that less august body of dispensers who in the late 18th century styled themselves "Chemists and Druggists" and practiced as such.

THE APOTHECARY—1180 to 1606

Early authentic records of the apothecary in England are rare. While mention is made of an apothecary to Henry II (1180) there is, except for occasional references, no consecutive record till about the 14th century when the apothecaries were associated with the Pepperers (later called the Grossarii or Grocers), a guild trading in drugs and spices from the Near East, who are mentioned in the Pipe Rolls of 1180. From 1290 (when Henry Montpellier was apothecary to Queen Eleanor) Royal Court apothecaries were regularly appointed. Between 1329 and 1360 several of these are named, notably Pierre de Montpellier, Apothecary to King Edward III. Chaucer in the celebrated "Canterbury Tales" refers to a Doctor of Physic who had apothecaries to supply drugs and electuaries.

Although barbers and surgeons had been formed into guilds during the 14th and 15th centuries it was not until the beginning of the 16th century in the reign of Henry VIII that an attempt was made to regulate by statute the practice of medicine in England and Wales.

In 1511 was enacted a law which for the avoidance of the "grievous hurt, damage and destruction of many of the people" allowed no person to practice as a physician or surgeon either in the City of London and environs or elsewhere within the Realm unless he were first examined by the Bishop of London (or the Dean of St. Paul's) and four doctors of physic and experts in surgery or by the Bishop of the Diocese or his Vicar-General and expert persons.

"This set up one body of practitioners who practised Medicine, Surgery and Pharmacy. The Physicians' assistants were styled Apothecaries and they gradually acquiring information respecting the properties of drugs, began to transact business on their own account." (Jacob Bell.)

In 1518 Thomas Linacre, physician to King Henry VIII, was instrumental in securing the incorporation of the physicians in a "College of Physicians." Its powers were extended by a Charter of 1540, authority being given to "search, view and see the Apothecary wares, drugs and stuffs" and to destroy those which were unfit for use.

The surgeons had been incorporated in one Company (Guild) with the barbers in 1467, when their respective duties were defined. They, having meanwhile abused their powers, were censured in the Act of 1542, which at the same time authorized irregular practitioners who had been freely treating the poor with simple remedies to continue administering external medicines and simples.

That there was growing up a class of traders tending to make the selling of drugs and medicines their principal concern, whether styling themselves apothecaries or not is shown by the Act of 1533 enlarging the physicians' powers to "govern, correct and punish Physicians, Apothecaries, Druggists, Distillers and sellers of waters and oils, and preparers of *chemical* medicines." The records show that some unqualified practitioners were arrested; one who persisted in practice had his ears cut off. Between this date and 1600, physicians who had themselves prepared and compounded their medicines began to give up this work, relying more and more on apothecaries or grocers specializing in drugs.

THE SOCIETY OF APOTHECARIES—1617 TO 1841

Early in the reign of King James I (1606) the apothecaries were incorporated with the grocers in a company (Guild). This proved unsatisfactory to the apothecaries, if not also to the grocers. The King was petitioned by a number of influential physicians and

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apothecaries and in 1617 he granted the apothecaries a separate Charter of Incorporation as a Company (Guild) which shortly afterward became known as the *Society of Apothecaries* (the only Company in the Guilds of London to be known as a Society).

The year 1617 is a landmark in the history of pharmacy in Great Britain. Henceforward it was intended the specialist in the preparation of medicines whether of animal, vegetable or mineral orgin should be set apart, his practice regulated and his obligations to the community fixed. Though many turns and twists were to come in the succeeding centuries, even a divergence of ways—the apothecary to practice medicine and to forsake pharmacy—yet the course was set and there was no looking back.

It is worth while spending a little time on the Charter granted by King James on the petition of "Theodore de Mayerne[1] and Henry Atkins, Doctors of Physic—our discreet and faithful Physicians . . . to promote the state of our Commonwealth and to procure the Public Good, that the ignorance and rashness of Presumptious Empirics and ignorant unexpert men may be restrained"—so runs the charter. No less than 114 apothecaries were charter members of the Company.

No surgeon was to sell medicines and no grocer to be allowed to keep an apothecary's shop or to compound and administer medicines or to exercise "the Art, Faculty or Mystery of an Apothecary" within the City of London and seven miles thereof.

Apprentices to apothecaries were to serve seven years, then to be examined (by apothecaries and a physician) in their knowledge and election (choice) of simples, and the preparing, dispensing and compounding of medicines. If approved the apprentice might then set up an apothecary's shop.

An important right and duty of *search* was laid on the Master and Wardens of the Society to search shops, cellars, etc., of any apothecary or others and to survey and test whether drugs offered were fit for the purpose or not. They were to prohibit from practice unfit persons and to burn unwholesome or improper drugs before the offender's door and fine the offender. (They could invoke the aid of the magistrates for this.) The rights of physicians (including that of search) were not curtailed although surgeons were restricted to supplying external medicines and salves and were not to sell these as the apothecaries did.

Notwithstanding the charter and the privileges conferred on Freemen of the Society a number of apothecaries remained outside and as time went on they became a source of vexation to the Society. Some of these free-lances desired reincorporation with the grocers—which the Society stoutly resisted. Indeed, turning the tables they proceeded to take action against those grocers who continued to sell drugs.

Soon a schedule was made of all medicines that should be stocked by apothecaries; apprentices were examined; and in 1620, the apothecaries' status improved by a proclamation which forbade the compounding of medicines in London except under the Society of Apothecaries and according to the London Pharmacopœia.

(It may be mentioned that the Colleges of Physicians of Edinburgh, Dublin and London issued separate pharmacopœias which remained in current use until 1864 when the first British Pharmacopœia was issued by the General Medical Council, set up under the Medical Act of 1858.)

Searches of drugs continued, the Master and Wardens of the Society starting out at 5 A.M. on set days for the purpose. "Herbor-

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[1] Theodore de Mayerne (Sir Theodore Turquet de Mayerne—but known in France as de Turquet) was born in Geneva 1573. He studied at Heidelberg and Montpelier and became first a lecturer to apothecaries in Paris, then a physician to Henri IV (under Riverus) where his protestantism finally brought him into disrepute and led to his dismissal. This is said to have been due mainly to Marie de Medici and the Faculty of Paris. On being invited to London his reputation caused him to be introduced to Court, when he became first physician to King James I and his Queen, then to Charles I, later to Charles II. He died in London in 1655 leaving a fortune of £140,000. He was a man of energy and was largely instrumental in getting the College of Physicians to publish the first London Pharmacopœia (1618) for which he wrote the dedication to King James I.

Another foremost apothecary of the time and a signatory to the petition for a charter, again trained in France, was Gideon de Laune who became Master of the Society, honored and revered by the craft so much that in 1675 his bust was set up in the Society's Hall.

isings" and "Simpling" (gathering of herbs locally) for the benefit of apprentices—and intended as botanical excursions—degenerated into holiday pursuits; the apprentices who had usually long hours and few holidays were admonished for this levity.

Toward the middle of the 17th century druggists, chiefly importers or wholesale dealers, became a loosely associated body sometimes styled the Company of Druggists but there is no record of their having been incorporated. Later, when the selling of chemicals became more general, merchants, both wholesale and retail, sold both drugs and chemicals, largely for the preparation of medicines and simples. These vendors became known as "Druggists" or "Chemists & Druggists." Many years were to pass, however, before the title "Chemist & Druggist" became legally protected and its use restricted to trained and qualified pharmacists.

As apothecaries became better trained and better able to distinguish between good medicines and those not so good, some commenced to give advice to patients and to prescribe medicines.[2] This may have been caused partly by the scarcity of physicians during the Plague (1660). It led to trouble with the physicians and was for years a thorny and oft-debated question. The physicians retaliated at first by charging the apothecaries with selling bad and unfit drugs; then they said they would do their own dispensing—some of them did so to the great hurt of the apothecaries' business—prescriptions sent to the apothecaries omitted dosage, which the physicians gave separately to their patients, but in the English language as their patients would not have understood Latin. For a time things settled down, the apothecaries agreeing not to prescribe and the physicians not to dispense, but the physicians felt they had a grievance when (in 1696) they found the apothecaries charging so much for made-up medicines and repeating the prescriptions so often that there were complaints that few people except the wealthy could "afford" to be ill.

The physicians decided to set up three dispensaries where the poor were to be assured of ample, cheap and proper medicine. This annoyed the apothecaries who said the physicians' drugs were bad and their dispensers unqualified, and that their own livelihood was threatened. To these dispensaries people came nevertheless to get their prescriptions dispensed and to buy medicines by retail.

This incensed the apothecaries who decided the public must be educated in the wisdom of buying medicines from those specially trained to handle them—how often in England is this plea to be heard!—and the apothecaries advertised their lengthy training, their scientific knowledge of drugs and chemicals and especially their fine Hall where "all may see the necessary processes of chemical preparations." For by this time (1700) a well-equipped "Elaboratory" had been set up, where all the galenical and many chemical operations were daily performed. Chemicals made there were to be sold only to physicians, surgeons, druggists and to those apothecaries who were members of the Society.[3]

They had just previously negotiated with Lord Cheyné of Chelsea for the purchase of Chelsea Physic Garden, which they had apparently held on lease, and where they conducted the "herborizings" or botanical walks and grew specimen drugs, herbs and fruits. Through Sir Hans Sloane (physician), a munificent benefactor to science and arts in this country—one of the principal founders of the British Museum—the Society acquired a perpetual lease of the Garden[4] in 1722 for £5 a year.

During the first half of the 18th century the Society, which had relied upon its mem-

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[2] The motto of the Society—"*Opiferque per Orbem Dicor*"—suggests their proper sphere in the early days was clearly considered to be that of physicians' assistants.

[3] About this time the Society obtained the privilege of supplying all medicines for the Fleet and established a special stock called "The Navy Stock." Later (1801) they supplied the East India Co. and in 1822 opened a retail department for supplying the public—this department was continued until 1922.

[4] The Physic Garden is still maintained by the voluntary subscriptions of a number of bodies including the Society of Apothecaries and the Pharmaceutical Society, the students of whom have the privilege of admission.

bers to train their apprentices prior to examination, made plans for giving systematic instruction. A Repository (Museum of Reference) for drugs and materia medica was formed, the institution of botanical lectures as well as lectures in materia medica and chemistry was considered, though it was not until about 1800 that these lectures formed part of routine training.[5]

In 1785 when a sixth edition of the London Pharmacopœia was under consideration—by the College of Physicians—the help of the apothecaries was invited and was readily given. The resulting volume in 1788 was distinguished by the inclusion of chemical medicines, foreshadowing the reliance to be placed upon the growing science of chemistry.

The previously mentioned attempts by the chemists and druggists to establish themselves side by side with the apothecaries could not be ignored by the latter—it was too severe an encroachment upon their legal privileges and livelihood. A determined inquiry was instituted throughout the country by an unofficial association of apothecaries, The General Pharmaceutical Association of Great Britain, and the illegal practices of the druggists condemned, stress again being laid on the classical education and training of the apothecaries opposed to the ignorance and nefarious ingenuity of their competitors. The chemists and druggists established themselves as dispensers of medicine, however, so much so that in 1802 the apothecaries sought aid from them in protesting against the Medicine Act, then passed, which made the selling of even common remedies subject to licenses, fiscal stamps and duties.

As the chemists developed dispensing so the apothecaries reverted to prescribing and to surgery, again bringing themselves, as may be supposed, into conflict with the physicians and surgeons. Medical reform became a burning question—it was the Age of Reform—and apothecaries, surgeon-apothecaries, accoucheurs, dispensing chemists and assistants wanted an Act passed, setting up a new medical body to examine and licence competent persons and, *inter alia*, "prohibit the practice of pharmacy . . . by uneducated persons." This at once brought all the established bodies into the field, physicians, surgeons and the Society of Apothecaries all being fearful of losing existing privileges or of assuming new responsibilities.

The chemists and druggists, having now formed an association to protect their interests, held meetings attended by many pharmacists whose names or those of their firms have become household words in pharmacy—thus William Allen, Fellow of the Royal Society (of Allen & Howard, later Allen & Hanbury), John Bell, John Savory (Savory & Moore) were all staunch supporters of the new cause.

Here, except to mention the apothecaries' regular courses of instruction in botany, materia medica and pharmacy and their examinations which later included medicine, surgery and midwifery, we have reached the parting of the ways. The apothecaries had long sustained the practice of pharmacy in Great Britain but the majority of them gradually veered toward the practice of medicine and surgery. The Society was confirmed as a licensing body under the 1858 Medical Act. It is still a flourishing corporation, its licenciates spread far and wide, and it remains one of the Companies (or Guilds) of the City of London. Save that the Society retained an examination for dispensers—not recognized as entitling to registration as a pharmacist—the apothecaries' preoccupation with pharmacy ceased.

THE PHARMACEUTICAL SOCIETY OF GREAT BRITAIN—1841 TO DATE

Our concern is now to follow the fortunes of the chemists and druggists in the establishment of the Pharmaceutical Society of Great Britain in 1841.

The prompt action taken by the principal chemists and druggists of London in 1813 was successful in preventing the passage of restrictive legislation; perhaps, more important, it knit them together and showed the need for corporate action. When a new Bill was introduced into Parliament by the

[5] *Note:* We have so far considered mainly developments in the London area—there are few records made accessible at present which give information about any show of interest in the sciences relating to pharmacy by the Universities of Oxford and Cambridge—and as yet the University of London was not established.

apothecaries which appeared to harm the chemists and druggists, the latter advertised in the newspapers their intended opposition, continued the meetings, took Counsel's opinion, and presented a petition to Parliament. The existence of the chemist and druggist (or "old" apothecary, as he was called; the "new" apothecary prescribed and visited patients) became recognized.

The next thing that called for attention was the widespread sale of poisons, especially of arsenic, by grocers, oilmen and indeed by all kinds of shopkeepers who had no knowledge or training in handling or storing them. Poisoning fatalities were numerous. The chemists and druggists pointed out the need for restricting the sale of poisons to trained persons who had knowledge of their properties and asked for legislation that should give proper protection to the public. It was not until 1851, however, that the Arsenic Act was passed.

In 1841 a Government Bill was introduced which threatened to restrain anyone from carrying on the trade or practice of a chemist and druggist unless he was licensed, the license to be renewed annually. The Bill also would have made it an offense for a chemist to bandage a finger or to recommend a dose of black draught. All chemists and druggists, their assistants and apprentices were to be controlled by a Medical Council on which they were to have no representation—entirely contrary to the English idea of fairness. The principal chemists from all parts of the country met to protest against this. Reports were sent to every member of the drug trade, a committee was set up and petitions against the Bill presented to Parliament. The object aimed at was the safeguarding of the chemists' and druggists' rights.

A few of the committee led by John Bell, of London (father of a more distinguished son, Jacob Bell), saw further than this. They realized that what prevented real recognition of their value to the community was the absence of proper qualification—to be attained only by systematic education. The outcome of further meetings was a proposal that in their permanent interests chemists and druggists should form a Society to be called the Pharmaceutical Society of Great Britain to benefit the public, to elevate the profession of pharmacy and to assist the needy.

On the April 15, 1841, at the Crown & Anchor Tavern in the Strand, London, was held the now historic meeting at which the Pharmaceutical Society of Great Britain was formed, there being over 100 signatories. The first elected president was William Allen, F.R.S., chemist, pharmacist and philosopher, who among his widespread interests gave the time to this work. The Society quickly became known—by the end of 1841 it had about 800 members and associates and in a year's time there were nearly 2000. Regulations were framed and examiners appointed for the first examinations in 1842. Both the College of Physicians and the Senate of London University gave their blessings but declined to coöperate in the examinations.

In January 1842 rooms were taken at 17, Bloomsbury Square. A petition for a Charter of Incorporation, to secure both public and royal approval, was later presented to the Crown; this was granted on February 18, 1843. The avowed objects of the Society were to advance chemistry and pharmacy, to promote the education of pharmacists, to protect their interests, and to grant benevolence to needy members, their widows and orphans.

The affairs of the Society were in the hands of a President, Vice-President and Council—21 in all. Jacob Bell, one of the most distinguished members, organized scientific meetings, made known the aims of the Society up and down the country and commenced (in 1841) the monthly issue of the *Pharmaceutical Journal*. (The Journal was continued as a privately owned publication until his death in 1859 when the copyright in it passed under his Will to the Society, which to this day continues its circulation as a weekly journal.)

The lamentable state to which pharmacy had fallen was shown by the lack of books on the subject. The London Pharmacopœia, still in Latin, contained much that needed revision by pharmacists. Systematic teaching was soon to come. The Society ap-

pointed professors of botany, chemistry, materia medica—the celebrated Dr. Pereira—and pharmacy. Lectures were crowded and classes grew rapidly when in the newly established School of Pharmacy there was set up a laboratory for practical instruction in chemistry and pharmacy. The enlarged laboratory of 1845, the first of its kind in England, was adopted as the pattern for others. Scientific papers appeared in the *Pharmaceutical Journal* (with Jacob Bell as editor) and the discussions they provoked stimulated interest in the professional and scientific side of pharmacy. Mr. Peter Squire (President, 1859, later editor of Squire's *Companion to the British Pharmacopœia*) was associated with the first use of ether in operations in England (1864) and shortly afterward with the newly tried anesthetic—chloroform.

The Society assisted in the production of the first British Pharmacopœia—1864, first revision 1867—issued under the General Medical Council to supersede the separate pharmacopœias of London, Edinburg and Dublin. In all subsequent editions pharmacists whether as advisers or joint editors have assisted. The present secretary of the Pharmacopoeial Commission is the distinguished pharmacist (and physician), Dr. C. H. Hampshire.

As the need for protecting the health of the people became better understood the wrongful use by unqualified and untrained persons of titles suggesting qualification in pharmacy was suppressed. The Pharmacy Act of 1852 required the Pharmaceutical Society to maintain a Register of persons qualified to be registered as Pharmaceutical Chemists or Pharmaceutists and of those who were members of the Society, of Assistants and Apprentices. The examinations were confirmed including knowledge of the Latin language, botany, materia medica and of pharmaceutical and general chemistry—a separate Board of Examiners to be appointed for Scotland.

The outstanding Act, however, was that of 1868. It regulated the sale of poisons and altered the previous Pharmacy Act—a truly British foible, to compress two measures into a single Act. Far better had the two been kept separate for their combination at this date has beclouded pharmacy legislation ever since.

The selling or compounding of poisons in open shop was restricted to pharmaceutical chemists and to chemists and druggists—a newly examined class, formerly styled assistants. Henceforth no unqualified and unexamined person (except certified as already in business as a pharmacist) could practice as a pharmacist. For the successful passage of the Act Jacob Bell, who had become a Member of Parliament, worked untiringly. A number of pharmacists who formerly had complained of the Society's actions and had formed an association of their own were reconciled because of the protection obtained under this Act.

Pharmacists suffered two setbacks in 1908. A new Act authorized the sale of agricultural and horticultural poisons by shopkeepers who should be licensed in their municipalities, and limited liability companies were permitted to own pharmacies if they employed qualified pharmacists as superintendents in them. (This concession was largely due to the influence of the limited companies at a time when the views of Members of Parliament were especially favorable to such companies.) These companies who actually owned drug stores wanted the right to dispense and to sell poisons and to use the personal title, "Chemist." The effect of this concession is marked to-day, when two organizations of "Company Chemists" own between them no less than 1900 pharmacies—about 8% of the total number in Great Britain. Alas, there is no way back to the earlier phase of "one man, one shop," for so long discussed and, since this Act, so ardently desired!

We can only glance at the further growth of the Society, whose membership was still voluntary, but to whose efforts the pharmacist of to-day owes so much—higher professional standing; better education, linking him with the universities many of which have granted degrees in pharmacy. Thanks largely to the late Professor Henry Greenish the Society's School of Pharmacy became a School of London University (1924) and with the Pharmacological Research Labora-

tories is now incorporated as a college of the university, professors in the school of pharmacy becoming professors in the university.

One other development remains to be noticed—the Pharmacy and Poisons Act of 1933, the outcome of a four-year inquiry—1926 to 1930—by a government-appointed committee to inquire into the practice of pharmacy and the sale of poisons. Some of the fruits of this Act are being gathered; the full harvest is to come. In addition to regulating further the sale of poisons and the manufacture of medicines containing them, the Act now makes the entire profession self-governing, subject to its own inspectorate and disciples, with minimum official supervision.

There are now few pharmacists who are not members of the Society—at the end of 1939 membership exceeded 24,500; about 600 apprentices enter the profession yearly. Two years' pupilage or apprenticeship is the minimum, with three academic years' training in botany, chemistry, zoology, pharmaceutics, pharmacognosy and physiology, the object being either the attainment of a university degree (B.Sc. or B.Pharm. = licencee) or qualification as a Pharmaceutical Chemist (3 years' training) or (2 years) Chemist and Druggist.

Education and practice in Great Britain has long since overflowed into all the British Dominions and Colonies were pharmaceutical societies and legislation on English lines have come into being.

Thus far has the practice of pharmacy progressed from the days of the Apothecaries Guilds and the unorganized Chemists and Druggists of the 18th century. Next year the Centenary of the Pharmaceutical Society will be celebrated. But for the war it would have been a royal celebration in the Society's new building now being completed in Brunswick Square, London, not far from the more familiar Bloomsbury Square which has housed pharmacy for almost a century.

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Book Reviews

Dissertation on *Solidago virga aurea L.*—a monograph based on research carried out for the Ph.D. degree at the University of Hamburg by ROLF GNEKOW of Wandsbek, near Hamburg, June 25, 1938.

This thesis of 100 pages divided into 8 parts begins with an introduction and is followed by sections on botany, systematic arrangement, habitat, growth, morphology, anatomical description, parts of the plant, germination and development. Synonyms, commercial distribution, etymology, etc., are discussed. The chemical part embraces the results of investigation of moisture, ash, tannin, ethereal oil and other constituents. Other subjects considered are extraction of the drug, history, pharmacology, therapeutic preparations, prescription uses and other combinations. Ten pages of illustrations show the structure of plant parts. The references might have been somewhat extended by including the U. S. Pharmacopœia of 1850, 1860 and 1870, and references to uses by early settlers in the United States and to the substitutions which were made when there were shortages of more commonly used drugs.—E. G. E.

Fruit Pectins. Their Chemical Behavior and Jellying Properties, by C. L. HINTON. Department of Scientific and Industrial Research (Great Britain), Food Investigation Special Report No. 48. vii + 96 pages. Chemical Publishing Co., Inc., 148 Lafayette St., New York, N. Y., 1940. Price, $1.75.

The material presented in this book is based on the work carried out by the staff of the British Association of Research for the cocoa, chocolate, sugar confectionery and jam trades. Twenty-five laboratory-prepared pectins from oranges, lemons, apples, gooseberries and strawberries by different extraction methods were studied and their physical and chemical properties reported on. Factors affecting the jellying powers of pectins are discussed as follows: chemical composition, effect of heating, changes caused by the action of pectase, effect of alkalies and acids, effect of salts and effect of extractive process. There are 13 figures, 42 tables and 45 references to the literature.—A. G. D.